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Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Support: JSPS grant 15H04603

Title: Regulation of non-coding RNA contributes to the complete cessation of cell proliferation of neuron-like cells

Authors: *T. IMAMURA[1], N. YAMAMOTO[2], K. AGATA[3], K. NAKASHIMA[1];
[1]KYUSHU UNIV, Fukuoka-Shi, Japan; [2]Hoshi Univ., Tokyo, Japan; [3]Gakushuin Univ., Tokyo, Japan

Abstract: Neurons do not proliferate once their fate is determined. This is because if they proliferated, newly generated neurons might cause electronic noise that would disturb the already existing finely tuned neuronal cell network. Similarly, de-regulation of cell cycling mechanisms in cells other than neurons may increase the risk of tumor formation. In order to avoid such de-regulation, genomic DNA that defines genes involved in the cell cycling is set appropriately according to given environmental contexts. In addition to the genes that encode proteins, next generation sequencing technique allows to find a large group of “non-coding RNAs (ncRNA)” transcribed from genomic DNA. We have found that bidirectional promoters are the major source of gene activation-associated ncRNA. PC12 cells offer an interesting model for understanding the mechanism underlying bidirectional promoter-mediated cell cycle control. Nerve growth factor (NGF)-stimulated PC12 cells elongate neurites, and are in a reversible cell-cycle-arrested state. In contrast, these cells irreversibly differentiate and cannot re-enter the normal cell cycle after NGF plus cAMP treatment. In this study, using directional RNA-seq, we found that bidirectional promoters for protein-coding genes with promoter-associated ncRNA (pancRNA) were enriched for cAMP response element consensus sequences, and were preferred targets for transcriptional regulation by the transcription factors in the cAMP-dependent pathway. A spindle-formation-associated gene, *Nusap1* and *pancNusap1* were among the most strictly co-transcribed pancRNA-mRNA pairs. This pancRNA-mRNA pair was specifically repressed in irreversibly differentiated PC12 cells. Knockdown (KD) and overexpression experiments showed that *pancNusap1* positively regulated the *Nusap1* expression in a sequence-specific manner, which was accompanied by histone acetylation at the *Nusap1* promoter. Furthermore, *pancNusap1* KD recapitulated the effects of cAMP on cell cycle arrest. Thus, we conclude that pancRNA-mediated histone acetylation contributes to the establishment of the cAMP-induced transcription state of the *Nusap1* locus and contributes to the irreversible cell cycle exit for terminal differentiation of PC12 cells.

Disclosures: **T. Imamura:** None. **N. Yamamoto:** None. **K. Agata:** None. **K. Nakashima:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Support: KAKENHI Grant Numbers 23700406

Title: Role of Meis1 in the cerebellar development

Authors: ***T. OWA**, M. HOSHINO, S. TAYA;
Dept. of Biochem. and Cell. Biol., Natl. Inst. of Neurosci. NCNP, Kodaira, Japan

Abstract: Although there have been many studies on the development of cerebellar granule cells, its molecular machinery is still elusive. We found that Myeloid ecotropic viral integration site 1 (Meis1) was strongly expressed in all cells in the granule cell lineage during development, including mitotic granule cell precursors (GCPs) in the outer external granule layer (EGL) and posto-mitotic granule cells (GCs) in the inner EGL, molecular layer (ML) and inner granule layer (IGL). We generated Meis1 conditional knockout mice by crossing with Atoh1-Cre-Tg line, where *Meis1* gene was specifically deleted in the granule cell lineage. In Meis1$^{fl/fl}$; Atoh1-Cre-Tg mice, the size of the cerebellum was smaller than that of wild type littermates. The structure of the cerebellum was disorganized. The cKO cerebella have multiple small lobuli. We occasionally observed ectopic EGL, (clusters of mitotic GCPs) in the ML and/or IGL, that expressed Atoh1. We suspect that those ectopic EGL may be account for generating extra tiny lobuli. These results suggest that Meis1 is involved in cerebellar granule cell development. We are now crossing Meis1 $^{fl/fl}$ mice with other Cre driver lines, which will be also introduced.

Disclosures: **T. Owa:** None. **M. Hoshino:** None. **S. Taya:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.03/A3

Topic: A.01. Neurogenesis and Gliogenesis

Title: Pax6d is necessary and sufficient for retina lineage specification

Authors: *Y. TAO[1], J. CAO[2], S.-C. ZHANG[3];
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Abstract: Pax6 is required for the specification of multipotent retinal progenitors. However, the role of Pax6 isoforms in retinal development is not defined. By creating human embryonic stem cell (hESC) lines that lack isoform-specific PAX6 and those that inducibly express specific PAX6 isoforms under the full PAX6 knockout background using CRISPR/CAS9, we examined the roles of PAX6 isoforms in retinal progenitor specification from human embryonic stem cells (hESCs), which mimics the human retinal development process in vitro. Human ESCs with complete deletion of PAX6 fails to enter into the retina fate and generate photoreceptor cells. Cells with PAX6 isoform d (PAX6D) knockout exhibit the same phenotype as the full knockout while pax6 a/b isoform knockout shows normal retina lineage specification. Furthermore, inducible expression of PAX6D rescues the capacity of Pax6 full knockout cells to produce retinal cells. Ectopic expression of PAX6D in neural progenitor cells results in generation of retinal cells. Our study has thus revealed that PAX6D is necessary and sufficient for retina lineage specification.

Disclosures: **Y. Tao:** None. **J. Cao:** None. **S. Zhang:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

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Program#/Poster#: 491.04/A4

Topic: A.03. Stem Cells and Reprogramming

Title: Elucidation of neural stem cell character of caudal cell mass in developing chick embryo

Authors: *E.-S. LEE[1], J. LEE[1], S. KIM[1], H. PARK[1], J. PHI[2], S.-K. KIM[2], Y. HWANG[1], K.-C. WANG[2];
[1]Seoul Natl. Univ., seoul, Korea, Republic of; [2]Div. of Pediatric Neurosurg., Seoul Natl. Univ. Children's Hosp., seoul, Korea, Republic of

Abstract: Caudal cell mass (CCM) has been known to be the main player in secondary neurulation forming the secondary neural tube. This suggest CCM may have the character of neural stem cell (NSC). However, evidence of CCM's character as NSC has not been shown. The neural potential of CCM was assessed by confirming *in vitro* culture of neurospheres from CCM throughout the stages of secondary neurulation (Hamburger and Hamilton (H-H) stages 12 to 32). We further evaluated whether there was spatiotemporal diversity in the neural potential of the developing central nervous system by quantification of the *in vitro* neurosphere culture results from the brain, upper spinal cord, lower spinal cord, and CCM from various stages of development.
CCM was capable of in vitro neurosphere formation, which were able to differentiate into neuron, astrocyte, and oligodendrocyte, and able to self-renew. This provided evidence that CCM had characteristics of NSC. When the quantitative outcome of neurosphere formation from CCM of various stages was evaluated, the number of cultured neurosphere was greatest at H-H stage 28 for CCM. For brain, the greatest number of neurospheres was formed at stage 16. Considering the trend of increase in the number of neurospheres for brain, spinal cord, and CCM, the neural potential seemed to follow a cephalo-caudal direction as development proceeded.
This study showed that neurospheres can be cultured *in vitro* from CCM of various stages, supporting its character as NSC. The spatiotemporal diversity of NSC character was also shown, reflecting the dynamic process of neurulation during development.

Disclosures: E. Lee: None. **J. Lee:** None. **S. Kim:** None. **H. Park:** None. **J. Phi:** None. **S. Kim:** None. **Y. Hwang:** None. **K. Wang:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.05/A5

Topic: A.03. Stem Cells and Reprogramming

Title: Different sources of neural stem cells have similar morphological phenotypes but differences in spontaneous spike and burst activity

Authors: *E. D. PETERSEN[1], O. V. LOSSIA[1], A. PAL[1], W. E. MEDENDORP[1], U. HOCHGECHWENDER[2];
[1]Neurosci., [2]Col. of Med., Central Michigan Univ., Mount Pleasant, MI

Abstract: Neural stem cells are characterized as self renewing cell populations with the ability to differentiate into the multiple tissue types of the central nervous system (CNS). These cells can differentiate into mature neurons, astrocytes, and oligodendrocytes. This category of stem cells has been shown to be an effective treatment for neurodegenerative diseases such as Parkinson's disease and following traumatic injury to the CNS such as traumatic brain injury, stroke, and spinal cord injury. Most treatment studies with neural stem cells in animal models use embryonic brain derived neural stem cells. This approach presents both ethical and feasibility issues for translation to human patients. Adult sources of tissue are a more practical source of stem cells for transplantation therapies in humans.
Some adult tissues such as bone marrow and adipose tissue contain discrete populations of multipotent and embryonic like stem cells. Of these stem cell populations, some are able to respond to neuronal growth factors and can be expanded *in vitro*, forming neurospheres analogous to cells harvested from embryonic brain tissue.
Here, we describe a method for the collection and culture of adipose tissue which results in a population of neural stem cells that are able to be expanded *in vitro* and be differentiated into neuronal-like cells. These adipose derived cells display a similar phenotype to those derived from embryonic sources. When differentiated into neurons, cells derived from adipose tissue show spontaneous spike activity which is similar in waveform to the embryonic brain derived cell lines but show higher frequencies of spiking and bursting activity.

Disclosures: E.D. Petersen: None. **O.V. Lossia:** None. **A. Pal:** None. **W.E. Medendorp:** None. **U. Hochgechwender:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.06/A6

Topic: A.01. Neurogenesis and Gliogenesis

Title: Analysis of Ski as a regulator of neural stem cell transcriptome dynamics

Authors: *A. GRISON[1], T. MUKHTAR[1], Z. KARIMADDINI[3], J. BREDA[2], K. ESCHBACH[4], D. IBER[3], E. VAN NIMWEGEN[2], C. BEISEL[4], V. TAYLOR[1], S. ATANASOSKI[1];
[1]Dept. of Biomedicine, [2]Biozentrum, Univ. of Basel, Basel, Switzerland; [3]Computat. Biology, D-BSSE, [4]Quantitative Genomics Unit, D-BSSE, ETH, Zürich, Switzerland

Abstract: During cortical development many genes and signaling pathways are active but their functions and interconnections are poorly understood. The protooncogene Ski is a central integrator of signal and transcriptional activities through its interactions with different partners. In order to assess the role of Ski, we are comparing the dynamics of various signaling pathways in the presence and absence of Ski during cortical developmental. We use in vivo transgenic marking of Ski-deficient neural stem cells (*Hes5::GFP; Ski*$^{-/-}$) and committed neuronal progenitors (*Tbr2::GFP; Ski*$^{-/-}$) to identify, isolate (FACS sorting), and analyze the cells by RNA-Seq. Analyses of the sequencing results revealed distinct patterns of misregulation of pathways in the absence of Ski over time. Moreover, ISMARA (Integrated System for Motif Activity Response Analysis) allowed us to identify the transcription factors that show divergent activity profiles in Ski Wt and Ski KO neural stem and committed progenitor cells at given time points.
Additionally, to evaluate the cell autonomous effects of Ski in progenitor cells, we are performing *in utero* electroporation experiments knocking-down or over expressing Ski at different stages of development.

Disclosures: **A. Grison:** None. **T. Mukhtar:** None. **Z. Karimaddini:** None. **J. Breda:** None. **K. Eschbach:** None. **D. Iber:** None. **E. van Nimwegen:** None. **C. Beisel:** None. **V. Taylor:** None. **S. Atanasoski:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.07/A7

Topic: A.01. Neurogenesis and Gliogenesis

Support: Wellcome- DBT India Alliance Grant number 500197/Z/09/Z

Title: Mechanistic insights into regulation of the neuron-glia cell fate switch in the developing hippocampus by transcription factor Lhx2

Authors: *B. MURALIDHARAN[1], B. ROY[1], M. KERUZORE[2], D. PARLIER[2], U. MAHESHWARI[1], S. PRADHAN[3], K. KARMODIYA[3], L. D'SOUZA[1], S. GALANDE[3], E. BELLEFROID[2], S. TOLE[1];
[1]Dept. of Biol. Sci., Tata Inst. of Fundamental Res., Mumbai, India; [2]Univ. Libre de Bruxelles, Brussels, Belgium; [3]IISER, Pune, Pune, India

Abstract: Regulation of the neuron-glia cell fate switch is a critical step in central nervous system development. Previously, we showed that LIM-HD transcription factor Lhx2 is a

necessary and sufficient regulator of this process in the developing hippocampus. Loss of Lhx2 causes premature astrogliogenesis, and overexpression of Lhx2 prolongs neurogenesis (Subramanian et al., 2011). The mechanism of Lhx2 action is unknown.
To identify genome-wide targets of Lhx2 at the onset of hippocampal neurogenesis, we performed ChIP-sequencing using E12.5 hippocampal primordia. We identified 6.4T as a potential Lhx2 target. Mimicking Lhx2 loss and gain of function, conditional mutants of 6.4T display enhanced GFAP in the hippocampus, and 6.4T overexpression promotes neurogenesis and suppresses astrogliogenesis. Significantly, this function of 6.4T is preserved in the absence of Lhx2, indicating that 6.4T acts downstream of Lhx2 and is sufficient to rescue Lhx2 loss of function.
The Lhx2 binding site lies within an enhancer in the 3'UTR of 6.4T in a highly conserved region across xenopus, chick, mouse, and human. A fragment of this enhancer including the Lhx2 binding site is sufficient to drive expression of a reporter transgene selectively in the hippocampus. Together, our data suggest that the Lhx2-6.4T pathway may be part of an evolutionarily conserved mechanism in the hippocampus that suppresses astrogliogenesis until neurogenesis is complete.

Disclosures: **B. Muralidharan:** None. **B. Roy:** None. **M. Keruzore:** None. **D. Parlier:** None. **U. Maheshwari:** None. **S. Pradhan:** None. **K. Karmodiya:** None. **L. D'souza:** None. **S. Galande:** None. **E. Bellefroid:** None. **S. Tole:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.08/A8

Topic: A.03. Stem Cells and Reprogramming

Support: Secretaria de Educacion Superior Ciencia y Tecnologia Ecuador (SENESCYT)

Title: Identifying gene regulatory networks during human neural induction

Authors: ***L. DUTAN POLIT**[1], G. COCKS[1], L. W. STANTON[2], S. HAVLICEK[2], S. SUBRAMANIAM[3], M. GERSTEN[4], J. PRICE[1], N. J. BUCKLEY[5];
[1]Basic and Clin. Neurosci., King's Col. of London, London, United Kingdom; [2]Stem Cell and Regenerative Biol., Genome Inst. of Singapore, Singapore, Singapore; [3]Bioengineering, Chem. and Biochemistry, Cell. and Mol. Medicine, Nano engineering, [4]Dept. of Bioengineering, UCSD, California, CA; [5]Dept. of Psychiatry, Univ. of Oxford, Oxford, United Kingdom

Abstract: Neural induction is the earliest event in the formation of the human nervous system. Identifying the gene regulatory networks that govern this process is fundamental to our understanding of the establishment of neuronal phenotype and is also essential to our ability to manipulate human IPSCs to produce neural stem cells (NSCs) specified toward particular neuronal fates. Human iPSCs readily undergo neural induction in the presence of inhibitors of BMP signaling toward NSCs fated to an anterior forebrain fate. We have found that inclusion of inhibitors of canonical WNT signaling switches specification of the NSCs to an olfactory placodal fate, destined to produce GnRH-positive hypothalamic neurons. Transcriptome data was captured at multiple time points during 8 days of neural induction. We have used these data to identify gene expression signatures unique to NSCs specified to these alternate fates. We are currently modeling these data to produce gene regulatory networks and use them to infer pathways that drive specification of neural stem cells toward to anterior cortical or olfactory placodal fates.
Key words: Olfatory placode, GnRH neurons, anterior forebrain, gene regulatory network.

Disclosures: L. Dutan Polit: None. **G. Cocks:** None. **L.W. Stanton:** None. **S. Havlicek:** None. **S. Subramaniam:** None. **M. Gersten:** None. **J. Price:** None. **N.J. Buckley:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

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Program#/Poster#: 491.09/A9

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH NINDS Grant ZIA NS002824-26

Title: Embryonic neurogenesis in the olfactory placode

Authors: *N. C. WHITTINGTON, S. WRAY;
NINDS, NIH, Bethesda, MD

Abstract: Recent evidence indicates that during embryonic development, neural crest and ectodermal cells contribute to the olfactory placode and give rise to olfactory sensory neurons, gonadotropin-releasing hormone (GnRH) cells, and olfactory ensheathing cells. However the mechanisms by which these initial neuronal fates are established and regulated in the olfactory placode have yet to be examined. The present work seeks to identify the gene regulatory network that governs embryonic olfactory neurogenesis. Sox proteins have been shown to play important regulatory roles in neurogenesis in the central nervous system and olfactory placode. Sox2 cooperates with cofactors to regulate stem cell renewal and differentiation during development,

and is a component of the molecular pathway utilized in nasal placode induction. Sox21, a Sox2 target and binding partner, has been implicated in regulating differentiation, and has recently been shown to control the progression of neuronal differentiation in the central nervous system based on its level of expression. Although these Sox proteins appear to have roles in controlling neuronal differentiation, their coordinated functions in the olfactory placode have not been explored. To determine whether Sox21 may have a role in regulating olfactory neurogenesis, the spatiotemporal expression of Sox21 is being analyzed in the developing mouse. RT-PCR and immunocytochemistry data confirmed Sox21 expression in the neurogenic regions of the nasal pits at E10.5-E11.5. At these ages, the olfactory placode consists of a multitude of cell populations, including neural stem cells, neuronal progenitors, neuronal precursors and neurons. To determine which cells express Sox21, sections were immunostained with markers identifying cells in these different stages of neurogenesis. Sox21 was expressed in progenitor and mitotic cells, and was absent in neuronal precursors, immature neurons, and GnRH cells. Thus, the expression pattern of Sox21 is consistent with it having a regulatory role in the developing olfactory placode. To understand the function of Sox21, its expression will be manipulated using in vivo and in vitro methods, and progression of neurogenesis in the olfactory placode followed. This work will delineate neurogenesis in the olfactory placode, and define the regulatory network involved in a cell's decision to stay as a progenitor cell or differentiate.

Disclosures: N.C. Whittington: None. **S. Wray:** None.

Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH F32 NS074742

NIH K99 MH104595

NIH MH071670

NIH NS08297

NIH NS0749729

NIH MH095147

Title: RETROSPECT: A strategy to timestamp the transcriptome of neural progenitors to identify fate determining genes

Authors: *T. J. PETROS, J. DIMIDSCHSTEIN, G. FISHELL;
New York Univ., New York, NY

Abstract: A fundamental impediment to furthering our understanding of brain development is the ability to track changes in gene expression in specific neuronal populations over time. GABAergic interneurons provide an ideal and particularly relevant context for exploring methods to overcome these issues. Interneurons are an extremely heterogenous population of cells that arise from two transient structures in the ventral telencephalon, the medial and caudal ganglionic eminences (MGE & CGE), and migrate tangentially to occupy numerous forebrain structures. However, the genetic programs that regulate these fate decisions and maturation into distinct interneuronal subclasses remain largely unknown. The lack of early markers that distinguish different interneuron subgroups within the MGE and CGE, combined with the inability to relate gene expression in progenitor populations with the mature interneurons they give rise to, has greatly hindered progress in this area. The technique of DNA adenine methyltransferase identification (DamID) represents one extremely promising avenue to overcome these challenges. We aim to 'timestamp' the transcriptome of MGE interneuron progenitors as they undergo fate decisions embryonically while retaining the capacity to identify their interneuron subclass in the mature brain. The creation of a spatially and temporally inducible Dam protein fused to RNA polymerase will allow us to methylate actively transcribed genes within differentiating MGE progenitors for the retrospective identification of fate determination genes in mature interneuron populations. Of note, this strategy is applicable for transcriptomes in any cell type, particularly ones in which mature markers of specific cell types are not present during earlier developmental timepoints.

Disclosures: **T.J. Petros:** None. **J. Dimidschstein:** None. **G. Fishell:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.11/A11

Topic: A.01. Neurogenesis and Gliogenesis

Title: A tracer system for lifelong neural stem cell fate with lentivirus.

Authors: *S. ISHIDA[1], A. TANAKA[1], T. FUCHIGAMI[1], Y. FUKAZAWA[2], S. HITOSHI[1];
[1]Shiga Univ. of Med. Sci., Otsu, Japan; [2]Univ. of Fukui, Yoshida-gun, Japan

Abstract: In mammalian brains, each neural stem cell (NSC) progeny divides several times as a neural progenitor, differentiates into a post-mitotic cell, and migrates into a specific region. It is controversial if cells derived from NSCs in the perinatal period migrate to a single or multiple region(s) and when cells become fated to migrate to specific sites. To answer these questions, we have focused on cell lineage of lifelong NSCs in the perinatal mice's brains, and established a system to trace the lifelong NSC lineage with lentivirus.
At embryonic day (E) 16 or postnatal day (P) 0, mice's brains were infected intraventricularly with lentivirus that expresses GFP and the mice were sacrificed at P30. Tissues were collected from cortex (Ctx), olfactory bulb (OB), and subependymal zone. Neurosphere assay was conducted with cells from the subependymal and GFP positive neurospheres were collected after passaging twice or more, which we regarded as cells generated from lifelong NSC. DNA was extracted from Ctx, OB, and the neurospheres. After a lentivirus integration site in genomic DNA of each neurosphere line was identified by an inverse PCR technique, PCR was done with DNA extracted from Ctx and OB as templates and primers corresponding to this lentivirus integration site. Then, electrophoretic pattern was analyzed to trace cell lineage from lifelong NSC. Our analysis with this system indicated that (1) major portion of NSC lines did not produce cells migrating to any of Ctx or OB, thus these cell lines were thought to be quiescent, (2) minor portion of the cell lines promoted cells migrating to exclusively wither of Ctx or OB, thus we did not identify a cell lineage migrating to two regions, and (3) among lifelong NSCs of the perinatal brain, symmetric and expansive division was rare.

Disclosures: S. Ishida: None. **A. Tanaka:** None. **T. Fuchigami:** None. **Y. Fukazawa:** None. **S. Hitoshi:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

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Topic: A.01. Neurogenesis and Gliogenesis

Support: National Natural Science Foundation of China 31530091

National Natural Science Foundation of China 91232304

National Natural Science Foundation of China 81571188

Title: Phosphofructokinase-1 negatively regulates neurogenesis from neural atem cells

Authors: *D.-Y. ZHU, F.-Y. ZHANG, X.-D. QIAN, C. QIN, Y.-H. LIN, H.-Y. WU, L. CHANG, C.-X. LUO;
Nanjing Med. Univ., Nanjing, China

Abstract: Neural stem cells (NSCs), present in both the developing and adult nervous system, could be an ideal therapeutic tool for neurodegenerative disorders, if they differentiate into the optimal types and numbers of neurons. Phosphofructokinase-1 (PFK-1) is a rate-limiting enzyme in glycolysis by phosphorylating fructose-6-phosphate to form fructose-1, 6-bisphosphate, and has been implicated in the functions of astrocytes and neurons. Here, using loss- and gain-of-function approaches, we showed that PFK-1 negatively regulates neurogenesis by mediating the proliferation of neural progenitors and the neuronal fate commitment of NSCs. Using in vitro assays, we found that PFK-1 knockdown enhanced, and PFK-1 overexpression inhibited the neuronal differentiation of NSCs, which was consistent with the findings from NSCs subjected to 5 h of hypoxia. Meanwhile, the neurogenesis induced by PFK-1 knockdown was attributed to the increased proliferation of neural progenitors and the commitment of NSCs to the neuronal lineage. Similarly, in vivo knockdown of PFK-1 also increased neurogenesis in the dentate gyrus of the hippocampus. Finally, we demonstrated that the neurogenesis mediated by PFK-1 was likely achieved by targeting mammalian achaete-scute homologue-1 (Mash 1), neuronal differentiation factor (NeuroD), and sex-determining region Y (SRY)-related HMG box 2 (Sox2). All together, our results reveal PFK-1 as an important regulator of neurogenesis.

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Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.13/A13

Topic: A.01. Neurogenesis and Gliogenesis

Support: R01AA024659

Title: Calcium dynamics in neural stem cells after chronic ethanol exposure and withdrawal

Authors: *A. H. MAHNKE[1,2], R. C. MIRANDA[1,2];
[1]Neurosci. and Exptl. Therapeut., [2]Women's Hlth. in Neurosci. Program, Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Ethanol is a known teratogen and *in utero* exposure can lead to a spectrum of neurodevelopmental disorders known collectively as Fetal Alcohol Spectrum Disorders. The end of the first trimester to the beginning of the second trimester is a particularly sensitive time to ethanol exposure, since during this time stem cells rapidly divide to produce most of the adult neurons. Previously, we showed that ethanol exposure during this period alters the programming of murine neural stem cells to favor premature maturation rather than renewal. Here, we further examined how ethanol exposure at sub-binge, binge, and chronic drinking levels (60mg/dL, 120mg/dL, and 320mg/dL) affects active cellular dynamics of gestational day 12.5 murine neural stem cells in neurosphere cultures. Cultures were chronically treated with ethanol for 5 days and then imaged, or allowed to withdraw from ethanol for two days before imaging. After treatment, cells were loaded with a calcium indicator dye, Fluo-4 AM, and imaged on a confocal microscope. Fluo-4 fluorescence allowed for the visualization of calcium dynamics in individual cells and showed that cells within an individual neurosphere are heterogeneous with respect to calcium dynamics. Some cells maintain steady calcium levels, while other cells exhibit dynamic calcium activity. Preliminary data suggest that these dynamics can be altered by both ethanol exposure and withdrawal. Notably, the mid-range, 120mg/dL dose was particularly effective at altering calcium dynamics in the neurosphere, increasing the number of cells exhibiting calcium oscillations as well as increasing overall frequency and amplitude of calcium spikes. After the withdrawal period, 120mg/dL treatment appears to have a lasting influence on calcium dynamics, with previously exposed cells showing an increased amplitude of calcium signal. These data firstly indicate the emergence of significant and surprising cell-to-cell variability in calcium activity among daughter fetal neural progenitors within a neurosphere niche that originate from a common stem cell. Moreover, these data also show that ethanol exposure continues to perturb cellular dynamics, even after ethanol is withdrawn, which may contribute to prolonged alterations to calcium-dependent intracellular signaling pathways that control neural stem cell growth and maturation.

Disclosures: **A.H. Mahnke:** None. **R.C. Miranda:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.14/B1

Topic: A.01. Neurogenesis and Gliogenesis

Title: Potential effect of melatonin on induction in neuronal markers in amniotic mesenchymal stem cells

Authors: *R. PHONCHAI[1], T. PHERMTHAI[2], B. CHETSAWANG[3];
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Abstract: Melatonin, an endogenous neurohormone mainly secreted by pineal gland in the brain, has several biological functions including antioxidative and neuroprotective activity. Additionally, melatonin is able to enhance embryonic brain cells proliferation and differentiation. However, its potential effect on neuronal differentiation has never been investigated in amniotic fluid mesenchymal stem cells (AF-MSCs). In the present study, we isolated stem cells from amniotic fluid, characterized mesenchymal stem cell marker antigens and then examined the cytotoxic, proliferative and differentiative effects of melatonin in AF-MSCs. After cells expansion in culture medium for 6-8 passages, the melatonin-induced neuronal differentiation in AF-MSCs was investigated using alpha-MEM with or without melatonin. Melatonin at 0-1,000 nM were not caused cytotoxicity and not increased proliferation in the cells. Western blot analysis showed increase in neuronal protein (βIII-tubulin, TH, and Nurr1) expression with dose-dependent effect in melatonin-treated cells whereas GFAP expression was suppressed by melatonin. Flow cytometry analysis demonstrated that melatonin increased nerve cell adhesion molecule (NCAM) and TH expression and changed differential expression patterns of mesenchymal stem cell antigens (CD29, CD73, CD90, CD105, CD34 and CD45). These results suggest that melatonin is able to potentiate amniotic fluid stem cells differentiation into neuronal cells observing by increase in neuronal protein markers and decrease in surface marker antigens of mesenchymal stem cells.
Keywords: Melatonin, amniotic fluid mesenchymal stem cells, differentiation, dopaminergic neuron

Disclosures: **R. Phonchai:** None. **T. Phermthai:** None. **B. Chetsawang:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.15/B2

Topic: A.01. Neurogenesis and Gliogenesis

Support: Interdisciplinary Program for Biomedical Science

Title: RNA-binding protein MARF1 regulates embryonic neurogenesis.

Authors: *Y. KANEMITSU[1], M. FUJITANI[2], Y. FUJITA[1], T. YAMASHITA[1];
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Abstract: Autism is a disorder of brain development characterized by impaired social interaction and communication. The latest child autopsy studies suggest the abnormality of neuronal fate specification during pregnancy. Microduplication in Chromosome 16p13.11 is one of known risk factors of autism. We hypothesize that one or several candidate genes in 16p.13.11 locus could be responsible for neurogenesis and pathophysiology of autism. After expression screening by *in situ* hybridization in mouse developing brain, we examined the function of RNA binding protein Meiosis Arrest Female 1 (MARF1). It has been already reported that oocyte form of MARF1 (oMARF1) suppresses the target molecules via its own RNase activity, and is indispensable for oogenic process. However, the function of another isoform, somatic form of MARF1 (sMARF1) is still obscure. Here we report the expression pattern and function of sMARF1 in developing mouse brain. sMARF1 protein is highly expressed in embryonic brain tissue and more in matured differentiated neurons than cortical pogenitors. The overexpression of sMARF1 in neuronal precursor cells which is derived from embryonic day-12.5 cortices, increases the number of Tuj1-positive differentiated neurons, while the knockdown of sMARF1 increases the number of Ki67-positive proliferating cell *in vitro*. The knockdown of sMARF1 *in vivo* by *in utero* electroporation increases the ratio of proliferating cell and Pax6-positive radial glia in subventricular zone. Moreover, we show that sMARF1 promotes neuronal differentiation in vitro via RNase activity domain. Thus, our findings suggest that sMARF1 regulates neuronal differentiation in developing brain through RNase activity.

Disclosures: Y. Kanemitsu: None. **M. Fujitani:** None. **Y. Fujita:** None. **T. Yamashita:** None.

Poster

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Location: Halls B-H

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Program#/Poster#: 491.16/B3

Topic: A.01. Neurogenesis and Gliogenesis

Support: Institutional Funding

Title: A complex of the ubiquitin ligase TRIM32 and the deubiquitinase USP7 balances the level of c-Myc ubiquitination and thereby determines neural stem cell fate specification

Authors: *S. NICKLAS[1], A.-L. HILLJE[1,2], S. OKAWA[3], I.-M. RUDOLPH[2], F. COLLMANN[2], A. DEL SOL[3], J. C. SCHWAMBORN[1,2];

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Abstract: Stem cells are able to self-renew and to undergo differentiation. The balance between stem cell maintenance and differentiation has been proposed to depend on antagonizing ubiquitination and deubiquitination reactions of key stem cell transcription factors. Based on this hypothesis, the stability of such stem cell transcription factors is controlled by one or more pairs of E3 ubiquitin ligases and deubiquitinating enzymes. Accordingly, increased ubiquitination of the stem cell transcription factor results in its proteasomal degradation, thereby inducing cellular differentiation, whereas increased deubiquitination stabilizes the stem cell transcription factor leading to maintenance of the stem cell fate. In neural stem cells, one of the key stem cell transcription factors is c-Myc. Previously, we have shown that c-Myc is ubiquitinated by the E3 ligase TRIM32 during embryonic and adult neurogenesis, thereby targeting c-Myc for proteasomal degradation and inducing neuronal differentiation. Consistent with its role in inducing neuronal differentiation, we have shown that TRIM32 becomes upregulated during adult neurogenesis, as neural stem cells progress towards a more differentiated fate. This upregulation is accompanied by a subcellular translocation of TRIM32 from the cytoplasm of neuroblasts within the rostral migratory stream to the nucleus of olfactory bulb neurons. However, we observed that a subpopulation of proliferative type C cells in the subventricular zone already contain nuclear TRIM32. As these cells do not undergo neuronal differentiation, despite containing TRIM32 in the nucleus where it can ubiquitinate c-Myc, we hypothesize that antagonizing factors, specifically deubiquitinating enzymes, are present in these particular cells. Here, we show by using a mass spectrometry approach that TRIM32 associates with various deubiquitinating enzymes, including USP7. This particular deubiquitinating enzyme is of special interest, since it has been implicated in neural stem cell maintenance before. USP7 and TRIM32 were found to exhibit a dynamic and partially overlapping expression pattern during neuronal differentiation both *in vitro* and *in vivo*. Most importantly, we are able to demonstrate for the first time that USP7 deubiquitinates c-Myc, thereby antagonizing TRIM32 function. Thus, we provide a potential mechanism by which stem cell fate is determined in neural stem cells, namely through the balanced ubiquitination and deubiquitination of c-Myc by TRIM32 and USP7.

Disclosures: **S. Nicklas:** None. **A. Hillje:** None. **S. Okawa:** None. **I. Rudolph:** None. **F. Collmann:** None. **A. del Sol:** None. **J.C. Schwamborn:** None.

Poster

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Program#/Poster#: 491.17/B4

Topic: A.01. Neurogenesis and Gliogenesis

Support: DFG GRK1657

BMBF NeuroRad

Title: Effects of ionizing radiation on neuronal stem cells during differentiation

Authors: K. RAU, B. ROTH, *B. LAUBE;
TU Darmstadt, Darmstadt, Germany

Abstract: In the developing and adult brain the exact cellular proliferation, migration, and differentiation mechanisms of neural stem cells (NSCs) are of prime importance. Ionizing radiation (IR) affects neurogenesis in the dentate gyrus of the hippocampus, which may explain learning deficits observed in patients treated with radiotherapy. NSCs are characterized by the ability of self-renewal and to differentiate to neurons, astrocytes and oligodendrocytes. Increasing evidence suggests that the activity of receptors and ion channels is intimately related to the control of proliferation and differentiation into defined cell types. Here, we have studied the physiology of early differentiation and network formation in neuronal cultures derived from the murine neural stem cell line J1 in a 2D differentiation protocol. Upon differentiation, the J1 cells show a loss in the stem cell marker nestin and a specific expression of certain differentiation markers and synaptic proteins like GFAP, MAP2 and PSD95, synaptophysin, respectively. Based on this differentiation protocol, we selectively irradiated (up to 0,5 Gy) individual differentiation stages and investigated the effects of IR on differentiation events like synaptogenesis, migration or neuronal functionality. Since potassium channels regulate cell behaviors such as proliferation and migration through both canonical ion permeation-dependent and noncanonical ion permeation-independent functions, we initially assessed the properties and composition of potassium channels expressed in different developmental stages upon radiation. By using whole-cell patch-clamp recording and immonohistochemistry, we measured the current responses of irradiated and unirradiated J1 NSCs in self renewal state and determined the potassium conductance of undifferentiated and differentiated J1 cells. We can show that IR increase the conductance of potassium channels, indicating that IR has lasting effects on the bioelectronical settings of NSCs. Thus, we can show that even low dose radiation of neural stem cells leads to significant variations in its electrophysiological homeostasis and the biophysical properties (like potassium conductance) of NSCs. In conclusion, NSCs could be successfully differentiated into functioning neural networks with differential developmental patterns upon radiation that may lead to subtle deficits in neuronal function.

Disclosures: **K. Rau:** None. **B. Roth:** None. **B. Laube:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

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Topic: A.01. Neurogenesis and Gliogenesis

Support: AA013440

AA024659

Title: miRNA-pseudogene interactions as a regulator of neural stem cell pluripotency and a target for ethanol teratogenesis

Authors: ***N. SALEM**, S. BALARAMAN, R. HOLGATE, E. RAYMOND, R. C. MIRANDA; Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Fetal alcohol exposure is a leading non-genetic cause of neurodevelopmental disability. Neural stem cells (NSCs) that give rise to most neurons of the adult brain during the first and second trimester are particularly vulnerable. We previously found that ethanol exposure did not result in NSC death, but rather, the loss of NSCs due to premature maturation. Moreover, we found that a class of small non-protein-coding regulatory microRNAs (miRNAs) was decreased following ethanol exposure. We recently found that the loss of miRNAs result in expression of a network of genes that support premature NSC maturation. However, the question that remains is whether ethanol also specifically prevents NSC renewal by interfering with miRNA-regulated processes. To address this question, we assessed the regulation of the homeobox transcription factor, Oct4/POU5F1, which is important for maintaining stem cell renewal and pluripotency. The Oct4 family includes a number of transcribed non-protein-coding pseudogenes. We hypothesized that these pseudogenes serve as miRNA sponges. Immuno-precipitation studies with the miRNA binding protein, Ago-2, showed that one Oct4 pseudogene, Oct4PS9, bound miRNAs in both the nucleus and cytoplasm of NSCs, supporting its role as a miRNA sponge. RNA-capture analysis also showed that Oct4PS9 specifically associated with miRNAs. Our preliminary data shows that Oct4PS9 preferentially associates with miR-21, an ethanol-regulated miRNA which also localizes to both nucleus and cytoplasm of NSCs. These data suggest that Oct4PS9 may regulate miR-21 availability to the cytoplasmic RISC complex in addition to epigenetic pathways in the nucleus. Ethanol exposure resulted in an increase in expression of Oct4PS9 transcripts, but resulted in decreased expression of Oct4 protein. Pseudogene-miRNA interactions may protect pluripotency factors and consequently regulate

NSC renewal. These data advance a novel mechanism for ethanol teratology in that ethanol exposure may disrupt long non-coding RNA (lncRNA)-mediated regulatory mechanisms resulting in dysregulation of miRNA-function in fetal NSCs.

Disclosures: **N. Salem:** None. **S. Balaraman:** None. **R. Holgate:** None. **E. Raymond:** None. **R.C. Miranda:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.19/B6

Topic: A.01. Neurogenesis and Gliogenesis

Title: Neural differentiation of cultured human neuroblasts is associated with transcriptomic poly(A) tail length modulation

Authors: *D. J. KILTSCHEWSKIJ[1,2], T. BEILHARZ[3], M. J. CAIRNS[1,2,4];
[1]Mol. Neurobio., The Univ. of Newcastle, Callaghan, Australia; [2]Priority Res. Ctr. for Brain and Mental Hlth. Res., Hunter Med. Res. Inst., New Lambton, Australia; [3]Dept. of Biochem. and Mol. Biol., Monash Univ., Melbourne, Australia; [4]Schizophrenia Res. Inst., Sydney, Australia

Abstract: Differentiation of complex cell types involves multiple layers of posttranscriptional regulation to establish the appropriate patterns and timing of intracellular protein synthesis. One important dimension of this system is modulation of mRNA 3′ poly(A) tail length as this *cis*-acting structure is critical for mRNA stability and translation. Cleavage of this tail reduces both transcript cytoplasmic stability and potential for translation, and is therefore thought to be an important intermediate in gene repression by microRNA (miRNA) and the RNA-induced silencing complex. In the current study, we sought to characterize genome-wide changes in mRNA poly(A) tail length in retinoic acid differentiated SH-SY5Y cells and explore its relationships to steady-state mRNA levels. SH-SY5Y cells were subject to a 14 day sequential *all-trans* retinoic acid and brain-derived neurotrophic factor treatment regimen to obtain near-pure cultures of neuron-like cells. Transcriptomic and poly(A) tail changes were investigated and compared via high-throughput deep sequencing using conventional RNA-Seq and a novel high-throughput library preparation for genome-wide analysis of mRNA poly(A) tail length, termed PAT-Seq. After genome alignment, read-count and poly(A) tail length attribution, we identified differentiation-associated change in poly(A) tail length in over 1,700 mRNAs whereby a strong bias towards lengthening was observed. In addition, a striking correlation between mRNA expression and poly(A) tail length ($R^2 = 0.3064$, $p < 0.0001$) was discovered, lending support to existing research suggesting increased poly(A) tail length confers mRNA stability.

Differentiation-associated changes in poly(A) tail length also exhibited significant enrichment in neurogenesis and cell cycle gene ontologies, which was also observed in 5,600 differentially expressed genes ($p < 0.05$). These analyses provide compelling support for the role of poly(A) tail length dynamics in the posttranscriptional regulation of mRNA during neural development. We suspect that factors involved in the modulation of mRNA stability and translation also have significant role in the pathogenesis of disease.

Disclosures: D.J. Kiltschewskij: None. **T. Beilharz:** None. **M.J. Cairns:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

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Program#/Poster#: 491.20/B7

Topic: A.10. Development and Evolution

Title: CCP1 is a positive regulator of Notch signaling pathway in the regulation of neural stem cells

Authors: *J. KIM, D. HAN, S.-H. BYUN, M. KWON, K. YOON;
Sungkyunkwan Univ., SUWON-SI, Korea, Republic of

Abstract: In the developing central nervous system, it has been widely known that Notch signaling preserves progenitor pools, inhibits neurogenesis, and drives astrocyte differentiation. CCP1 is known as a chloride channel protein containing membrane spanning regions. *In situ* RNA analysis indicates that CCP1 is mainly transcribed in the ventricular and sub-ventricular zone (VZ and SVZ) in E14.5 embryonic mouse brain. In this study, we assessed the role of CCP1 in the mammalian brain development. We first found that Notch intracellular domain increased transcription of CCP1. Interestingly, CCP1 also synergistically increased transactivational ability of Notch. We used a retroviral vector system to investigate the effects of CCP1 expression on the properties of neural stem cells *in vivo* as well as *in vitro*. CCP1 induced efficient neurosphere formation, a high level of neural stem cell proliferation, and localization of neural progenitor population in the VZ and SVZ of developing mouse brain. Taken together, our data indicate that CCP1 is a novel Notch target gene and also a regulator in the canonical Notch signaling pathway.

Disclosures: J. Kim: None. **D. Han:** None. **S. Byun:** None. **M. Kwon:** None. **K. Yoon:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NSF Grant 1533708

NSF IGERT Training Grant in Neuroengineering, 1250104

Title: The social networks of neural progenitor cells

Authors: *A. S. MAHADEVAN[1], J. T. ROBINSON[1,2], A. A. QUTUB[1];
[1]Bioengineering, [2]Electrical and Computer Engin., Rice Univ., Houston, TX

Abstract: Objective: Cell-cell communication among neural progenitor cells (NPCs) is essential for proper self-organization of the nervous system, a topic of great interest in regenerative medicine. Graph theory and network analysis have been used to study the functional connectivity of mature neuronal circuits but not developing networks. In developing networks, different modes of cell-cell communication dominate than in mature neuronal networks, and proximity of cell bodies plays a key role in neural cell fate. The objective of this study is to quantitatively describe the spatial organization of progenitor cell bodies at different stages of neuronal differentiation in order to uncover the role of cell-cell communication during neural network formation.
Methods: hESC-derived neural progenitor cells were stimulated to differentiate through withdrawal of bFGF from culture medium and imaged continuously for up to 14 days. Time-lapse image sequences were processed using custom-written algorithms, and spatial proximity of cell bodies was used to create graph representations of NPC spatial topology. 16 graph-based metrics describing features such as connectivity, information flow and motif counts were evaluated in NPC graphs in order to quantify the spatial organization of cells at different time points. Functional maturation of NPCs was analyzed using whole-cell patch clamp electrophysiology and immunochemistry.
Results: Statistical measures of information flow in NPC spatial graphs revealed a shift from topologies with high global efficiency to high local efficiency, around the time mature neuronal phenotypes appeared in culture. Trends in information flow are intuitively explained by corresponding trends in graph connectivity. Our results support the view that network-wide signaling in immature progenitor cells gives way to more structured, hierarchical communication in mature neural networks. We also demonstrate that the evaluation of recurring motif patterns in NPC graphs reveals unique geometric arrangements of cells in neural rosette-like structures at early stages of differentiation.
Conclusions: Our method introduces a tangible means to test theories about spatially-dependent

forms of neural cell communication. Our results point to spatial re-organization of progenitor cells during electrical maturation, insights which help further our understanding of the design principles involved in the development of functional neural networks. Applications of this work can help pave the way for systematic modulation of neural cell self-organization for therapeutic purposes, eg. in treating repercussions of brain injury or stroke.

Disclosures: **A.S. Mahadevan:** None. **J.T. Robinson:** None. **A.A. Qutub:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

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Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R01NS048255

ANII FCE 2369

ANII FCE 100411

Title: Purinergic signalling in a latent stem cell niche of the rat spinal cord

Authors: ***N. MARICHAL**, G. FABBIANI, O. TRUJILLO-CENOZ, R. E. RUSSO, 11600; Neurofisiología Celular y Mol., Inst. De Investigaciones Biológicas Clemente E, Montevideo, Uruguay

Abstract: The ependyma of the spinal cord harbours stem cells which are activated by traumatic spinal cord injury. Progenitor-like cells in the central canal (CC) are organized in spatial domains with cells on the lateral aspects combining characteristics of ependymocytes and radial glia (RG), whereas in the dorsal and ventral poles they have the morphological phenotype of RG and display complex electrophysiological phenotypes. The mechanisms regulating the behaviour of these progenitors remain unknown. Because ATP is massively released after spinal cord injury we hypothesized that purinergic signalling may be important in this spinal stem cell niche.We combined immunohistochemistry, in vitro patch-clamp whole-cell recordings and Ca^{2+} imaging to explore the effects of purinergic agonists on ependymal progenitor-like cells in the neonatal (P1-P6) rat spinal cord. Prolonged focal application of a high concentration of ATP (1 mM) induced a slow inward current. Equimolar concentrations of BzATP generated larger currents that reversed close to 0 mV, had a linear current-voltage relationship and were blocked by brilliant blue G, suggesting the presence of functional P2X7 receptors. Immunohistochemistry showed that P2X7 receptors were expressed around the CC and the processes of RG. BzATP

also generated Ca^{2+} waves in RG that were triggered by Ca^{2+} influx and propagated via Ca^{2+} release from internal stores through activation of ryanodine receptors. Taken together, we showed that progenitor-like cells in the ependyma of the rat spinal cord have functional ionotropic P2X7 receptors. We speculate that the intracellular Ca^{2+} signalling triggered by P2X7 receptor activation may be an epigenetic mechanism to modulate the behaviour of progenitors in response to ATP released after injury.

Disclosures: **N. Marichal:** None. **G. Fabbiani:** None. **O. Trujillo-Cenoz:** None. **R.E. Russo:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant 1R01NS095734-01

Title: Regulation of neural stem cell division modes in the developing zebrafish brain

Authors: ***X. ZHAO**[1,2], R. CHOI[2], S. GUO[2];
[1]Neurosci. Center, U.Helsinki, Helsinki, Finland; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: Asymmetric cell division (ACD) is a conserved and fundamental process in neurogenesis for generating cellular diversity across both invertebrates and vertebrates. As the principal neural stem cells (NSCs), radial glia progenitors (RGP) undergo ACD to generate self-renewing and differentiating daughter cells in the developing central nervous system. Until now, the cellular and molecular mechanisms of ACD and subsequent daughter fate choice is not well understood in vertebrates. By using zebrafish, we have identified that the cortical polarity regulator Partitioning defective protein-3 (Par-3) plays a crucial role in the establishment of ACD through localizing the ubiquitin E3 ligase Mindbomb (Mib), which activates Notch by ubiquitinating the Notch ligand unequally in the apical daughter. Using immunocytochemistry, in vivo imaging, and other molecular genetic and biochemical methods, we are elucidating the nature of Mib asymmetry and the underlying mechanisms that orchestrate such asymmetry.

Disclosures: **X. Zhao:** None. **R. Choi:** None. **S. Guo:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant HDR01 27056-21

Howard Hughes Medical Institute

Title: *Drosophila* type II neuroblasts undergo temporal patterning

Authors: *B. MARK, M. H. SYED, C. DOE;
Biol., Univ. of Oregon, Eugene, OR

Abstract: The steroid hormone Ecdysone regulates *Drosophila* Type II Neuroblast temporal patterning.
How a small population of stem cells can give rise to neuronal diversity remains a fundamental question in developmental neuroscience. One mechanism by which stem cells have been shown to expand neural diversity is via temporal patterning programs. Stem cells change their expression of various transcription factors over time, conveying a unique transcriptional profile to progeny born in each window. Traditionally, these temporal patterning programs have been shown to be primarily cell intrinsic. Here we show that the insect steroid hormone ecdysone serves as an extrinsic cue that governs the temporal patterning program of *Drosophila* type II neuroblasts, a central brain stem cell with a transit amplifying division pattern similar to that of vertebrate outer subventricular zone progenitors. Ecdysone acts via its receptor EcR, a nuclear receptor which regulates transcription of a host of developmentally relevant genes. The ecdysone receptor EcR-B1 isoform is temporally expressed in type II neuroblasts beginning at early L3 larval stages and persists for the remainder of larval life. Ecdysone and its receptor EcR-B1 act together in order to induce the transition between two identity factors Chinmo and Broad-Complex, as well as inducing the expression of other transcription factors including E93, offering both a novel mechanism by which neuroblasts regulate temporal patterning, as well as a novel mechanism by which steroid hormones regulate development.

Disclosures: B. Mark: None. **M.H. Syed:** None. **C. Doe:** None.

Poster

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Program#/Poster#: 491.25/B12

Topic: A.01. Neurogenesis and Gliogenesis

Support: FARB 2015 Grant

Title: Bag3 is involved in neuronal differentiation

Authors: *S. L. NORI[1], V. NICOLIN[3], F. FLORENZANO[4], A. SANTORO[2];
[2]Dept of Medicine, Surgery and Dentistry, Scuola Medica Salernitana, [1]UNIVERSITY OF SALERNO, Baronissi, Italy; [3]Clin. Dept. of Medical, Surgical and Hlth. Sci., Univ. of Trieste, Trieste, Italy; [4]Confocal microscopy unit, Natl. Res. Council, European Brain Res. Inst., Rome, Italy

Abstract: BAG3 belongs to the family of co-chaperones interacting with the heat shock protein HSP70 through the structural domain known as BAG domain. BAG3 mediates the retrograde transport of both misfolded proteins to the microtubule organizer center (MTOC) and autophagic vacuoles to perinuclear region, facilitating the clearance of aggregated-prone proteins in several cell types. Some studies reported a BAG3 transient expression in rat cerebral cortex and hippocampus and a persistent expression in the rostral migratory stream and subventricular zone of the lateral ventricle. However, few data are reported on subcellular localization of BAG3 in neurons. From this point of view, we recently detected a molecular weight variant of BAG3 (40 kDA) in synaptosomes and evidenced that BAG3 mRNA is present in synaptosomal polysomes of rat brain. The objective of the present study was to provide further insights on the expression and subcellular distribution of BAG3 in neurons. We studied BAG3 expression in NGF-induced neurite outgrowth in PC12 cells, by using confocal and transmission electron (TE) microscopy and then performing morphometric analysis. Results showed that after NGF-induced neurite outgrowth, BAG3 localized mainly in the neuritic domain compared to cell body, while in control cells it appeared confined to the cytoplasm near the nuclear membrane. Interestingly this phenomenon was associated only to a slight increase in the total BAG3 immunofluorescence suggesting a change in its distribution rather than an increase in protein expression during neuronal differentiation. This observation was corroborated by TE microscopy analysis of NGF-treated PC12 cells showing that BAG3 was confined mainly in synaptic vesicles of the axon. Moreover, we also investigated BAG3 expression in the developing (E18) and adult cerebral cortex. Preliminary results indicate that BAG3 was intensely expressed in cellular processes and some migrating cells in the developing cortex, while in the adult cortex, only a low immunoreactivity was found in the majority of neuronal cell bodies and glial cells. Altogether

these data suggest that BAG3 could be involved in neuronal differentiation acting during the whole process.

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Poster

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Topic: A.01. Neurogenesis and Gliogenesis

Support: KMA

Medical Faculty Lund University

The Crafoord foundation

The Royal Physiographic Society of Lund

NanoLund

O. Engqvist Foundation

Title: Promoting neuronal differentiation and neurite guidance of human neural progenitors using electrospun PCL fibrous scaffolds

Authors: ***M. C. ZALIS**[1], S. JOHANSSON[1], F. JOHANSSON[2], U. E. JOHANSSON[3]; [2]Dept. Biology, Unit of Functional Zoology, Bio-interface Group, [3]Inst. Clin. Sci. in Lund, Dept. Ophthalmology, [1]Lund Univ., Lund, Sweden

Abstract: Transplanted neural cells show great potential to replace neurons in damage or disease where neurons are lost. However, limited long-term survival and functional outcome are yet major hurdles to overcome. Therefore, we hypothesized that the use of supporting artificial scaffolds may increase survival, neuronal differentiation and, neurite extension and guidance of neural progenitors. Here we initially studied the potential use of electrospun fibrous scaffolds for supporting the culture of human neural progenitors in vitro.
We fabricated biodegradable polycaprolactone (PCL) fibrous substrates, consisting of either randomly- and aligned oriented sub-micron fibers (fiber diameter: 550-700 nm). Human neural progenitor cells (HNPC), mitogen-expanded multipotent cell line, were cultured for 10 days on nanofibers and 2D (flat) glass surfaces. Survival, neurogenic potential, neurite extension and nuclei orientation, were studied using alamar blue assay, immunohistochemistry, and compared

to flat controls. Fluorescent- and scanning electron microscopy were used.
Excellent survival of HNPCs was found at both nanofiber scaffolds, closely comparable to flat surfaces controls. Morphological formation was strongly influenced by culture substrates, with significantly more multipolar morphologies found at random fibers and flat controls. At aligned fibers, a trend toward nuclei orientation in parallel with fiber orientation, more bipolar morphologies and longer neurites extended along the fibers were found. Significant increase in neuronal differentiation (MAP-2-positive cells) was found on nanofibers scaffolds compared to flat surfaces. In parallel, lower numbers of cells expressing markers of immature/glial cells, i.e. nestin-, S100b- and GFAP were found at both fibrous substrates.
In summary, PCL nanofiber scaffolds indicate biocompatibility, promote neuronal maturation of the HNPCs and influence neuronal morphological formation. The fact that neurons extended long neurites, a requirement for successful neuronal cell transplantation, and which can be guided by fiber orientation support the proposal of using scaffolds that can be tailor-made for advancing cell transplantation as a therapy.

Disclosures: **M.C. Zalis:** None. **S. Johansson:** None. **F. Johansson:** Other; Cellevate AB. **U.E. Johansson:** None.

Poster

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Program#/Poster#: 491.27/B14

Topic: A.01. Neurogenesis and Gliogenesis

Support: INPer 3230-21202-01-2015

Title: Effect of histamine H_1 receptor inhibition in rat embryo cortical and mesencephalic neuroepithelia: in the search for its functional role during fetal neurogenesis *In vivo*

Authors: *B. M. VALADEZ[1], N. F. DÍAZ[2], J. A. G. ARIAS MONTAÑO[3], A. MOLINA HERNÁNDEZ[2];
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Abstract: Histamine H_1 receptors (H_1Rs) are expressed in the rat neural tube from embryo day 14 (E14) to E20. Histamine reaches its highest level during the neurogenic peak (E14), and *in vivo* and *in vitro* experiments indicate its participation in neuronal differentiation through H_1R

activation. Histamine exerts differential affects on cerebral cortex and ventral mesencephalic neuroepithelia neural stem cells, promoting phenotype differentiation in cortex deep layers whereas in ventral mesencephalon reduces the tyrosine hydroxylase (TH) phenotype *in vitro* without affecting total immature neurons. In this study E12 and E14 rat embryos were used to study H_1R expression by RT-PCR, its membranal location by radioligand binding and its tissue distribution by immunocytochemistry. Because histamine has been proposed to participate in neuron commitment of neural stem cells, the main cell population at E12, the H_1R antagonist clorpheniramine was intraperitoneally injected at E12 and the deep layer cortical (FOXP2, Tbr1 and SOX5) and ventral mesencephalic (TH, lmx1b and PITX3) phenotypes were evaluated at E14 and E16 by immunohistochemistry. Our results show H_1R expression in both neuroepithelia at E12 and E14, its presence in total forebrain/midbrain membranes with a higher density at E12 and its location in the ventricular zone. H_1R inhibition negatively affects the cortical and mesencephalic markers at E14 and E16. These results indicate that the H_1R is expressed by neural stem cells in the cortical and ventral mesencephalic neuroepithelia with a likely membranal location, and that receptor blockade alters cortical and mesencephalic development, suggesting an important role of histamine and H_1Rs during brain development.

Disclosures: B.M. Valadez: None. **N.F. Díaz:** None. **J.A.G. Arias Montaño:** None. **A. Molina Hernández:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.28/B15

Topic: A.03. Stem Cells and Reprogramming

Support: National Science Foundation of China (81371384)

Special fund for experimental animals of Sshanxi Province[2014(06)] and [2014(k15)]

Title: The effect of decreased miR-31 expression on spinal cord stem cell transcriptomes

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Abstract: Spinal cord injury (SCI) often results in severe and permanent neurological deficits. Recent studies have shown that neural stem cell (NSC) replacement is effective in treating SCI.

However, one obstacle of this strategy is the low efficiency of NSC differentiation. Our previous study showed that the expression of miR-31 is significantly higher in spinal cord stem cells (SCSCs) isolated from embryonic day 14 (E14) spinal cord ependymal layer compared to motor neurons (MNs), indicating that miR-31 may play an important role in maintaining SCSCs in an undifferentiated state. In the present study, we characterize the RNA transcript profiles (transcriptomes) of SCSCs from E14 mice treated with small interfering RNA (siRNA) to lower the expression of miR-31. Total RNA was extracted from siRNA treated and non-siRNA treated E14 SCSCs (control) using TRIzol Reagent, and then sequenced on an Illumina HiSeq2500. Gene expression was analyzed with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). We found that 1295 genes were significantly differentially expressed. Among those genes, 549 were up-regulated and 746 genes were down-regulated in the SCSCs transfected with siRNA compared to control SCSCs. The further bioinformatics analysis using the GO classification shows that the molecular function of the differentially expressed genes mainly corresponds to channel regulator activity, receptor regulator, and chemoattractant activity, and the affected biological processes mainly are locomotion, biological adhesion, biological phase, hormone secretion and cell aggregation. The KEGG enrichment analysis showed that the pathways of differentially expressed genes mainly participate in focal adhesion, MAPK signaling, cytokine-cytokine receptor interaction, and neuroactive ligand-receptor interaction. Theses pathways are more related to neurons than stem cells. Our results demonstrate that down-regulation of the miR-31 expression can induce the differentiation of SCSCs, and that miR-31 plays an important role in the differentiation of SCSCs.

Disclosures: **C. Wang:** None. **P. Li:** None. **F. Tian:** None. **R. Zhang:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.29/B16

Topic: A.01. Neurogenesis and Gliogenesis

Title: Ets factors regulate gliogenesis in perinatal development and glioma

Authors: ***H. PARK**[1,2], R. LEVY[2], M. DUTRA-CLARKE[2], A. A. AKHTAR[2], G. B. KIM[2], M. DANIELPOUR[2], J. J. BREUNIG[2];
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Abstract: We have previously demonstrated that disruption of the Nf1-Ras pathway in the VZ compartment at multiple signaling nodes uniformly results in rapid NSC depletion, progenitor hyperproliferation, and gliogenic lineage restriction. Abrogation of Ets subfamily activity, which

is upregulated downstream of Ras, rescues these phenotypes and blocks glioma initiation. Thus, the Nf1-Ras-Ets axis might be one of the select molecular pathways that is a hijacked for initiation and maintenance in glioma. Herein, we examine the cell autonomous role of Ets proteins during perinatal development. Expression analysis and inducible lineage tracing indicates that Etv5 is enriched in glial subtypes. Using a novel genetic technology to independently misexpress several Etv5 variants (i.e. full-length, truncated, constitutively active) or loss-of-function elements (e.g. miR-E shRNA against Etv5), we observe phenotypes consistent with a putative role for Etv5 in cell fate determination of glia. Moreover, we present findings that the ability of Etv5 to block gliomagenesis can be generalized to other driver mutations. Thus, Ets proteins represent a key mechanism of glial cell fate determination in gliogenesis and glioma.

Disclosures: **H. Park:** None. **R. Levy:** None. **M. Dutra-Clarke:** None. **A.A. Akhtar:** None. **G.B. Kim:** None. **M. Danielpour:** None. **J.J. Breunig:** None.

Poster

491. Fate Specification and Neural Progenitor Biology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 491.30/B17

Topic: B.12. Glial Mechanisms

Support: NHLBI DIR

Title: Altering proteoglycans leads to unexpected changes in neural cells

Authors: ***C. MENCIO**, S. TILVE, C. AGBAEGBU, H. KATAGIRI, H. GELLER; NHLBI, Natl. Inst. of Hlth., Bethesda, MD

Abstract: Proteoglycans (PGs) are important to a variety of neurological functions. From neural development to memory to regeneration and disease, both heparan sulfate (HS) and chondroitin sulfate proteoglycans (CSPGs) play a number of promoting and inhibitory roles. While research is clear that these macromolecules are involved, their actual role of action remains relatively unclear. This is in part due to the lack of tools with which to study them and our incomplete understanding of the tools that are currently available. One class of these such tools are xylosides. Xylosides are small molecules consisting of an aglycone attached to a xylose residue which serve as a competitor for glycosaminoglycan (GAG) chain biosynthetic machinery. Used in research since the 1970s, treatment with these molecules leads to the inhibition of endogenous PGs and the production of primed GAGs, or GAG chains built on the xyloside and as such lack a core protein. Primed GAGs are pushed out of the cell and can be found in the extracellular space.

Much of the previous research has focused on high concentration treatment (≥ 1mM) by xyloside and largely bypassed any role primed GAGs may play in experimental outcomes. We have found that low concentration (≤ 1μM) xyloside (LCX) treatment will also alter the GAG profile of and PG production by astrocytes, neuro2A cells, and primary neurons. Reduction in CSPGs was seen in LCX samples at 72h after treatment, much later than observed in samples treated with higher concentrations of xyloside which showed significant reduction at 24 and 48h. Additionally, LCX treatment caused unexpected changes in cell behavior and morphology. LCX-treated neuronal cells demonstrate enlarged growth cones with microtubule looping that were absent in cells that were exposed to both vehicle and 1mM xyloside treatment. This research serves as a first step to fully explore the potential of xylosides as a research tool and possible therapeutic for injury and disorders of the nervous system.

Disclosures: **C. Mencio:** None. **S. Tilve:** None. **C. Agbaegbu:** None. **H. Katagiri:** None. **H. Geller:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.01/B18

Topic: A.01. Neurogenesis and Gliogenesis

Support: Wellcome Trust Research 103714MA

Title: Clonal analysis of the development of pyramidal cell lineages in the mouse cerebral cortex

Authors: A. LLORCA[1], G. CICERI[1], R. BEATTIE[2], C. STREICHER[2], S. ARNOLD[3], M. MARAVALL[4], S. HIPPENMEYER[2], *O. MARIN[1];
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Abstract: During the development of the mammalian cerebral cortex radial glia cells (RGCs) produce different layer-specific subtypes of excitatory pyramidal neurons. At the population level, these cells are generated in an inside-out pattern that is highly correlated with their birthdate. Classical models of cortical development propose that this phenomenon is due to the progressive restriction of RGCs to generate progressively more superficial layer neurons over the time, and experiments both in vitro and in vivo are consistent with this idea. However, recent studies have also suggested the existence of pools of RGCs in the rodent cortex with restricted potential to generate specific classes of pyramidal cells. In this study, we combine retroviral

lineage tracing and Mosaic Analysis with Double Markers (MADM) techniques with Cre/LoxP mouse genetics to trace the progenies of individual RGCs in the mouse developing cerebral cortex. By unbiasedly analysing the outcome of dozens of clones, we have obtained information about the relative contribution of different classes of RGCs to cortical development. Our results indicate that the most abundant progenitor cells in the rodent cortex have the potential to generate pyramidal cells for all cortical layers, but the diversity of RGCs or their potential is larger than previously recognised. This diversity of neural progenitors may play a prominent role in orchestrating the complex laminar organisation of the mammalian cerebral cortex.

Disclosures: **A. Llorca:** None. **G. Ciceri:** None. **R. Beattie:** None. **C. Streicher:** None. **S. Arnold:** None. **M. Maravall:** None. **S. Hippenmeyer:** None. **O. Marin:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.02/B19

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH T32 GM007752

NIH RO1 AA021402

Title: Whole genome single cell sequencing confirms the presence of mosaic aneuploidy throughout cortical development

Authors: *S. E. ROHRBACK[1], J. CHUN[2];
[1]Biomed. Sci., Univ. of California San Diego, La Jolla, CA; [2]Mol. and Cell. Neurosci., The Scripps Res. Inst., San Diego, CA

Abstract: Contrary to popular belief, not every cell in the body contains identical DNA. This phenomenon of genomic mosaicism was first identified as a driving force behind cancer, but has more recently been found to be enriched in the brain. In particular, aneuploidy has been shown to be enriched in cerebral cortical cells, most dramatically in neural progenitor cells (NPCs) during embryonic development. Despite being confirmed by several independent groups using cytogenetic methodologies, there is controversy regarding the rates and persistence of aneuploidy in the brain. To assess this discrepancy, we used a customized single cell sequencing and bioinformatics approach to obtain an unbiased, genome-wide assessment of the copy number states in a variety of sample preparations. Three novel insights have been gained from our work. First, during the development of our custom bioinformatic pipeline, we found that several

commonly used quality control measurements are biased towards excluding hypoploid cells from analysis**.** Eliminating their use increased the accuracy of aneuploidy rate assessments in control populations**.** Second, we developed a strategy to sequence the individual mouse metaphase spreads, which are the gold standard for determining cellular ploidy. This approach confirmed that single cell sequencing accurately identifies the occurrence of mosaic aneuploidy. Finally, we applied our single cell sequencing method on over 400 interphase nuclei collected throughout cortical development, and have confirmed that aneuploidy is both present in interphase NPCs and can persist in adult neurons. This pipeline is being utilized to gain further insights into neuronal aneuploidies during and beyond cortical development.

Disclosures: S.E. Rohrback: None. **J. Chun:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.03/B20

Topic: A.01. Neurogenesis and Gliogenesis

Support: JSPS KAKENHI Grant Number 15K18548

Title: Mechanisms that balance neuronal subtype production in the developing neocortex

Authors: *K.-I. TOMA, C. HANASHIMA;
RIKEN Ctr. For Developmental Biol., Kobe, Hyogo, Japan

Abstract: The cerebral cortex consists of diverse layer neurons that differ in their connectivity, morphology and molecular properties. These neurons are generated in a stereotypical temporal order during development, where neurons of deep-layer (DL) are generated first, followed by more superficial upper-layer (UL) neurons. While DL neurons establish long-range connections by sending axons to subcortical targets, UL neurons play central roles in higher order information processing by integrating bilateral cortical information. Despite their functional significance, the mechanisms by which laminar subtypes are generated at the correct timing and in appropriate numbers remain largely elusive.
Here, we employed a non-biased genetic ablation to eliminate early post-mitotic DL neurons in developing mouse neocortex. By using this method, we found that cortical progenitors produce DL neurons for a longer period to compensate for the loss of DL neurons, thereby maintaining the final ratio between DL and UL neurons. These results demonstrated that the mechanisms that adjust the number of DL and UL neurons required for proper circuit formation utilizes feedback signals propagated from DL neurons. We further explored the identity of this feedback signal

focusing on the molecular events that occurs during the switch in progenitor competence from DL neurons to UL neurons. Our results indicate that this feedback signaling controls the localization of UL neuron-specific transcription factors and determine the timing of neuronal differentiation. This feedback regulatory system confers the sequence of DL and UL neurogenesis, and scales the production of UL projection neurons based on the availability of their DL neuron counterparts during development and possibly evolution.

Disclosures: K. Toma: None. **C. Hanashima:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

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Program#/Poster#: 492.04/B21

Topic: A.01. Neurogenesis and Gliogenesis

Support: MEXT KAKENHI Grant Number 22123007

Grant-in-Aid for JSPS Fellows

Sasakawa Scientific Research Grant from The Japan Science Society

Title: Dmrt genes control the development of Cajal-Retzius cells derived from specific origins in the cerebral cortex

Authors: *T. KIKKAWA[1], N. SAKAYORI[2], H. YUUKI[1], N. OSUMI[1];
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Abstract: For the development of the central nervous system, a large variety of neuronal cell types are needed to be generated at defined times and locations. Cajal-Retzius (CR) cells are the first neurons generated during corticogenesis and are essential pioneer neurons that control neuronal migration in the cortex. CR cells are derived from specific regions within the cortex, i.e., the pallial septum (PS) in the rostromedial cortex, and the pallial-subpallial boundary (PSB) and cortical hem (CH) in the caudomedial cortex. However, the molecular mechanism underlying the generation of CR cell subtypes in distinct CR cell origins is poorly understood. We have previously shown that *Dmrta1* (*double-sex* and *mab-3* related transcription factor like family A1) is expressed in neural stem/progenitor cells in the neocortex and regulates proneural genes (Kikkawa et al., 2013). In this study, we found that Dmrta1 was expressed in the PS, PSB, and a part of the CH, whereas Dmrt3 was strongly expressed in the CH. To reveal functions of *Dmrta1* and *Dmrt3* in the production of CR cells from distinct origins, we observed CR cells in

Dmrta1 knockout (*Dmrta1*$^{-/-}$) (Kikkawa et al., unpublished) and *Dmrt3* knockout (*Dmrt3*$^{-/-}$) mice (Konno et al., 2012). We found that *Dmrt3* ablation decreased the number of p73-positive CR cells derived from the PS and CH compared with wild type (WT) mice, suggesting an abnormal medial-cortical structure of *Dmrt3*$^{-/-}$ and *Dmrta1*$^{-/-}$ *Dmrt3*$^{-/-}$ mice. On the other hand, *Dmrta1*$^{-/-}$ mice shows the reduction of p73-positive CR cells derived from the PS. There was no significance difference of the number of Calretinin-positive/p73-negative CR cells derived from the PSB. These results suggest that Dmrt members are differentially involved in the development of CR cells according to their original cortical regions.

Disclosures: **T. Kikkawa:** None. **N. Sakayori:** None. **H. Yuuki:** None. **N. Osumi:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

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Program#/Poster#: 492.05/B22

Topic: A.01. Neurogenesis and Gliogenesis

Support: MOST 104-2917-I-564 -007 -

Grant-in-Aid for Scientific Research 16H04798

Title: Temporal dynamics of laminar subtype neuron differentiation in developing mouse neocortex

Authors: *P. HOU, C. NISHIYAMA, C. HANASHIMA;
RIKEN Ctr. For Developmental Biol., Kobe-Shi, Japan

Abstract: The cerebral cortex holds a remarkable capacity to integrate multimodal sensory information and generate coordinated outputs that underlies higher cognitive functions. While the localization of neocortical areas responsible for modality-specific information processing has been mapped over a century ago, how each area is assembled to serve its unique function has remained largely elusive. To address this, we established a highly reproducible tracing system to label cohorts of neurons generated at distinct developmental timing utilizing the *Neurog2*-driven Cre recombinase. Upon tamoxifen administration, we specifically marked layer subtype neurons using various fluorescent reporter lines in vitro and in vivo. Based on this approach, we monitored the dynamics of neurons generated at different corticogenesis period. We further applied this system in culture to assess the capacity of neuronal differentiation of these cortical cells in vitro. These results indicated that laminar subtype identity are determined soon after the

cell cycle exit, providing a useful platform to assess the mechanisms underlying neuronal specification in cortical establishment.

Disclosures: **P. Hou:** None. **C. Nishiyama:** None. **C. Hanashima:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

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Program#/Poster#: 492.06/B23

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS081297

NRSA fellowship F32 NS074742

K99 Fellowship K99 MH104595

Title: Challenging interneurons in novel environments reveals roles for both intrinsic and environmental mechanisms that sculpt their maturation

Authors: *G. QUATTROCOLO, G. J. FISHELL, T. J. PETROS;
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Abstract: The majority of interneurons derive from two different embryonic structures: the Medial Ganglionic Eminence (MGE) and the Caudal Ganglionic Eminence (CGE). Distinct subtypes of interneurons originate from each structure, with Parvalbumin- and Somatostatin-expressing interneurons deriving from the MGE, and Reelin- and VIP-positive interneurons deriving from the CGE. Postmitotic interneurons are partially fate determined, and they go on to populate numerous regions within the telencephalon. Recent clonal analysis (Mayer et al., 2015; Harwell et al., 2015) has showed that the same progenitor cell in the MGE can give rise to interneurons located in different structures, such as the cortex, hippocampus or striatum. In addition, it was shown that both Parvalbumin- and Somatostatin-expressing interneurons can derive from the same progenitors. These data seems to exclude the presence of different progenitors pools giving rise to cortical versus hippocampal interneurons. However, little is known regarding the mechanisms guiding the interneurons maturation and their integration in distinct neuronal networks. Which aspects are intrinsically encoded and which ones are dependent on environmental cues? To address this question we isolated the cortex and the hippocampus of Nkx2.1-Cre;Ai9 mice at P0-P2, sorted the fluorescent cells and transplanted them either homotopically (cortex-to-cortex) or heterotopically (cortex-to-hippocampus, cortex-to-striatum) to P0-P2 wild type mice. A month after transplantation we analyzed the molecular,

electrophysiological and morphological profile of the transplanted interneurons. The data suggest that: 1) the heterotopic environment can sculpt the maturation of the transplanted interneurons as they integrate in the network, and 2) different interneuronal features are inherited from the donor environment. Our findings shed light on the mechanisms regulating the interneuron development and neuronal circuit formations.

Disclosures: G. Quattrocolo: None. **G.J. Fishell:** None. **T.J. Petros:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.07/B24

Topic: A.01. Neurogenesis and Gliogenesis

Support: NS045523

NS075672

NS049553

NS080343

NS064730

Title: Ctip1 controls area-specific subtype ratios and subsequent differentiation in sensory cortex

Authors: *L. F. CUSTO GREIG[1], M. B. WOODWORTH[2], J. D. MACKLIS[2];
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Abstract: The molecular linkage between neocortical projection neuron subtype and area development, which enables the establishment of functional areas by projection neuron populations appropriate for specific sensory and motor functions, is poorly understood. Here, we report that Ctip1 controls precision of neocortical development by regulating both subtype identity and area identity in neocortical projection neurons. Ctip1 is expressed by postmitotic callosal and corticothalamic projection neurons, but is excluded over embryonic development from corticospinal motor neurons, which instead express its close relative, Ctip2. Loss of Ctip1 function results in a striking bias in favor of subcerebral projection neuron development in sensory cortex at the expense of corticothalamic and deep-layer callosal development, while misexpression of Ctip1 in vivo represses subcerebral gene expression and projections.In parallel,

Ctip1 functions in primary sensory areas to repress motor and activate sensory gene expression programs, enabling establishment of sharp molecular boundaries defining functional areas. In Ctip1 mutants, abnormal gene expression leads to aberrantly motorized corticocortical and corticofugal output connectivity. Ctip1 critically regulates differentiation of layer IV neurons, and selective loss of Ctip1 in cortex deprives thalamocortical axons of their receptive "sensory field" in layer IV, which normally provides a tangentially and radially defined compartment of dedicated synaptic territory. Therefore, although thalamocortical axons invade appropriate cortical regions, they are unable to organize into properly configured sensory maps.Together, these data identify Ctip1 as a critical control that couples subtype and area specification, enabling specific functional areas to organize precise ratios of appropriate output projections.

Disclosures: L.F. Custo Greig: None. **M.B. Woodworth:** None. **J.D. Macklis:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

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Program#/Poster#: 492.08/B25

Topic: A.01. Neurogenesis and Gliogenesis

Support: EMBO ALTF 1295-2012

NS 081297

MH095147

P01NS074972

Title: Clonally related forebrain interneurons disperse broadly across both functional areas and structural boundaries

Authors: *C. MAYER[1,2], X. J. JAGLIN[2], R. C. BANDLER[2], C. STREICHER[3], C. L. CEPKO[4], S. HIPPENMEYER[3], G. FISHELL[2];
[1]Physiol. & Neurosci., New York Univ., New York, NY; [2]NYU Neurosci. Inst., Nyc, NY; [3]IST Austria, Klosterneuburg, Austria; [4]Departments of Genet. and Ophthalmology and HHMI, Harvard Med. Sch., Boston, MA

Abstract: The medial ganglionic eminence (MGE) gives rise to the majority of mouse forebrain interneurons. Here, we examine the lineage relationship among MGE derived interneurons using a replication-defective retroviral library containing a highly diverse set of DNA barcodes. Recovering the barcodes from the mature progeny of infected progenitor cells enabled us to

unambiguously determine their respective lineal relationship. We found that clonal dispersion occurs across large areas of the brain and is not restricted by anatomical divisions. As such, sibling interneurons can populate the cortex, hippocampus striatum, and globus pallidus. The majority of interneurons appeared to be generated from asymmetric divisions of MGE progenitor cells, followed by symmetric divisions within the subventricular zone. Altogether, our findings uncover that lineage relationships do not appear to determine interneuron allocation to particular regions. As such, it is likely that clonally related interneurons have considerable flexibility as to the particular forebrain circuits to which they can contribute.

Disclosures: **C. Mayer:** None. **X.J. Jaglin:** None. **R.C. Bandler:** None. **C. Streicher:** None. **C.L. Cepko:** None. **S. Hippenmeyer:** None. **G. Fishell:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.09/B26

Topic: A.01. Neurogenesis and Gliogenesis

Support: NYSCF C028128

The Rockefeller University

Title: Projection neuron class specification occurs post-mitotically in the subplate during human corticogenesis.

Authors: ***M. Z. OZAIR**[1], C. KIRST[2], B. VAN DER BERG[4], A. H. BRIVANLOU[3];
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Abstract: The six-layered neocortex is unique to the mammalian lineage and has undergone significant expansion in primates. Classic studies of mouse and primate corticogenesis have established the inside-out nature of layer formation during development, while more recent studies have highlighted several developmental adaptations exclusive to primates. These adaptations together enable production of large numbers of projection neurons (PNs) that are required to populate the primordium of the primate neocortex, namely, the cortical plate. There are various classes of PNs on the basis of their projection hodology. While recent work has emphasized the role of post-mitotic regulatory programs in specification of PN classes, how these programs are executed and whether they are cell autonomous or under the influence of

signaling pathways is currently unknown. The subplate (SP) is a transient zone underneath the cortical plate that is also greatly enlarged in primates and is known to be essential for formation of cortical circuits. The human SP has been shown to be uniquely enriched in genes that lie near human accelerated conserved noncoding sequences. However, the significance of SP enlargement and its contribution to early corticogenesis remain unknown. Using a combination of in vivo human fetal brain observations, novel genetic and computational tools for analysis, and in vitro modeling of corticogenesis, we show that the SP is a major contributor to cortical lamination and projection neuron class-specification. Our results establish that SP neurons represent a novel ground state upon which post-mitotic transcriptional refinement takes place and culminates in the major classes of PNs that populate the six layers. Furthermore, we provide direct evidence that the human SP derives from post-mitotic preplate neurons. We also demonstrate that the SP is the exclusive contributor to layers V-VI in parts of the developing cortex. In addition, our *in vitro* and *in vivo* experiments provides clues to signaling pathways that regulates these post-mitotic programs and in turn specify SP neurons to various PN classes. The relative ratios of PN classes are thought to be altered in psychiatric and neurodevelopmental disorders such as schizophrenia, autism, and intellectual disability. By establishing a link between SP fate and PN class specification, our work provides a potential link between SP and developmental disorders. Overall, these findings have strong implications for understanding human corticogenesis, neurodevelopmental diseases, disease modeling, as well as stem cell-based therapies.

Disclosures: **M.Z. Ozair:** None. **C. Kirst:** None. **B. van der Berg:** None. **A.H. Brivanlou:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.10/C1

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant MH071679

Title: Non-canonical Wnt signaling regulates the ratio of somatostatin- and parvalbumin-expressing cortical interneurons

Authors: ***M. MCKENZIE**[1,2], L. COBBS[1], G. FISHELL[1], E. AU[2];
[1]Smilow Neurosci., NYU Sch. of Med., New York, NY; [2]Pathology and Cell Biol., Columbia Univ. Med. Ctr., New York, NY

Abstract: Cortical GABAergic interneurons exhibit remarkable diversity in their intrinsic firing properties, subtype marker expression, layer organization, synaptic connectivity and morphology. The mechanisms underlying the generation of this diversity are largely unknown. We have identified a novel rostral-caudal Wnt gradient within the medial ganglionic eminence (MGE) that delineates the specification of the two main interneuron subtype classes. Caudally-situated MGE progenitors receive high levels of Wnt signaling and give rise to somatostatin (SST)-expressing cortical interneurons. Parvalbumin-expressing basket cells, by contrast, originate mostly from the rostral MGE where Wnt signaling is attenuated. Interestingly, canonical Wnt signaling through b-catenin is not required for this process. Wnt signals transmitted via nuclear translocation of the intracellular domain of the non-canonical receptor Ryk, however, are sufficient to drive interneuron progenitors to a SST fate. Inhibition of Ryk signaling by a function blocking antibody conversely decreases the production of SST positive interneurons. Graded Ryk gain of function experiments performed in mouse ES-derived cortical interneurons reveal a dose-dependent effect, suggesting Ryk signaling acts in a gradient.

Disclosures: **M. McKenzie:** None. **L. Cobbs:** None. **G. Fishell:** None. **E. Au:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.11/C2

Topic: A.01. Neurogenesis and Gliogenesis

Support: China National Natural Science Foundation 31171039

Title: Transcriptome analysis of the developmental dynamics of Cajal-Retzius neurons in the embryonic mouse neocortex.

Authors: *J. LI, L. SUN, X. YU, S. QI, X. PENG, Q. SHEN;
Tsinghua Univ., Beijing, China

Abstract: Cajal-Retzius (CR) cells, among the earliest-born neuronal population of the developing neocortex, are crucial for brain development. They are involved in regulating subsequent neuronal migration and laminar patterning of the cerebral cortex. Previously we identified Ebf2 as a molecular marker for cortical neurogenesis and CR neurons during neural development. Here, we used a transgenic mouse line that expresses EGFP in Ebf2-expressing cells to purify Ebf2+ cell population by FACS at different embryonic stages. Gene expression profiles of Ebf2+ and Ebf2- cell population were analyzed by next generation RNA-seq. Bioinformatics analysis revealed differentially expressed genes in Ebf2-GFP+ cells and Ebf2-

GFP- cells and temporal gene expression dynamics during early embryonic CR neuron development. We found that many known CR neuron-specific genes, such as Reelin and p73, are enriched in Ebf2+ cell population, consistent with their identity being CR neurons. Based on the dynamic gene expression pattern at different stages, CR neuron-enriched genes can be clustered into different groups, reflecting that specific sets of genes may regulate different aspects of CR neuron differentiation. We also identified a number of lncRNAs that are enriched in CR neurons, confirmed by in vitro and in vivo data. In addition, we found that the upstream regulatory regions of these lncRNAs were associated with active histone modifications by ChIP studies, indicating epigenetic regulation of CR gene expression. Overall, our work has identified genes that are related to early neurogenesis, particularly CR neuron identity, and will help understand the molecular mechanisms governing the developmental function of CR neurons.

Disclosures: **J. Li:** None. **L. Sun:** None. **X. Yu:** None. **S. Qi:** None. **X. Peng:** None. **Q. Shen:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.12/C3

Topic: A.01. Neurogenesis and Gliogenesis

Support: Children's Hospital Los Angeles Pre-Doctoral Research Career Development Fellowship

Title: Distinct layer six neuron subtypes employ Foxp2 and Met during cortical development

Authors: *R. J. KAST[1], H.-H. WU[2], P. LEVITT[2];
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Abstract: The extraordinary cellular diversity of the six layered cerebral cortex is critical for the highest levels of perception, cognition and motor control. To better understand how these functions emerge during development, it will be critical to determine how neuronal diversity is generated within each cortical layer and how individual cell types reach functional maturity. Toward this end, here we map the developmental expression of two important neurodevelopmental genes, Foxp2 and Met, onto discrete and largely non-overlapping cell types in layer 6. The majority of layer 6 excitatory projection neurons fall into 2 categories: corticothalamic and corticocortical neurons. Through neuroanatomical tracing and molecular profiling experiments, here we find that the transcription factor Foxp2 is enriched in

corticothalamic neurons and the receptor tyrosine kinase Met is selectively expressed by corticocortical neurons in layer 6. This information is particularly relevant given evidence implicating these genes in related, yet distinct disorders of higher cognitive function. Moreover, each of these genes has been associated with aspects of neural development that distinguish these two cell types. Ongoing experiments involving conditional mouse genetics seek to determine how Foxp2 and Met influence the development of these important layer 6 cortical circuit components.

Disclosures: R.J. Kast: None. **H. Wu:** None. **P. Levitt:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.13/C4

Topic: A.01. Neurogenesis and Gliogenesis

Support: Leibniz Award of the DFG

Neurocure Stipend

Böhringer Ingelheim Fonds

Title: Approaches to the quantification of polyploid neurons in rat cortex

Authors: *J. SIGL-GLÖCKNER[1], M. BRECHT[2];
[1]AG Brecht, Bernstein Ctr. For Computat. Neurosci., Berlin, Germany; [2]Humboldt University/ BCCN Berlin, Berlin, Germany

Abstract: Principal cell diversity is a central issue in the neurobiology of the cerebral cortex. We address this issue by examining DNA content of cells in rat sensory cortex. Using fluorescence microscopy, we have previously shown that soma and nuclear size and patterns of DAPI-(DNA)-fluorescence vary between groups of cells in rat cortex. Layer 4 cells and non-neural cells are of small and homogenous size, while layer 5 exhibits a remarkable size diversity of somata and nuclei and contains some very large neurons. We also measured integrated DAPI-(DNA)-fluorescence and counted chromocenters, spots of densely packed heterochromatic DNA. In comparison to layer 4 neurons and non-neural cells, integrated DAPI-(DNA)-fluorescence and chromocenter counts of layer 5 neurons were greater and more widely distributed. These results suggested that some very large neurons in layer 5 are polyploid, owning more than two homologous copies of each chromosome. Moreover these supposedly polyploid neurons were differently distributed across cortical sensory areas. To better understand whether polyploidy is a

novel generator of neuronal diversity in the cortex, we recently focused on methods to directly quantify DNA content. On the one hand, we apply flow cytometry to more rigidly measure fluorescent staining of DNA in the nucleus. Combining DAPI-(DNA)-staining with immunohistochemistry, we are able to differentially measure DNA content of live neural and non-neural nuclei. Similar to previous reports[1], preliminary results revealed that 3-5% of cortical neurons appear to be tetraploid, containing 4 sets of homologous chromosomes. However, flow cytometry requires the homogenization of brain tissue and therefore curtails any insight into the phenotype and location of supposedly tetraploid neurons. Therefore we currently focus on approaches to directly quantify chromosomes of identified cells in brain sections. First, antibody staining is used to distinguish different cell types. Second, chromosomes are labelled using fluorescent in situ hybridization of telomeres with a peptide nucleic acid probe. Finally fluorescent telomere spots are imaged using confocal microscopy and counted, whereby supposedly tetraploid neurons will have twice as many telomeres. However, since hybridization requires excessive digestion, the integrity of the tissue is often harmed preventing complete hybridization. Once these challenges are overcome, we aim to combine size measurements, chromocenter counts, flow cytometry and in situ hybridization to gain a better understanding of how polyploid neurons contribute to cell diversity in the normal vertebrate brain. [1]López-Sánchez et al., 2014

Disclosures: J. Sigl-Glöckner: None. **M. Brecht:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.14/C5

Topic: A.01. Neurogenesis and Gliogenesis

Support: Rafael del Pino Foundation

NIH grant 9500303955

Title: Distinct progenitor lineages contribute to neuronal diversity in layer 4 of the barrel cortex

Authors: *T. GUILLAMON-VIVANCOS[1,2], M. MEDALLA[2], W. A. TYLER[2], T. F. HAYDAR[2], J. I. LUEBKE[2];
[1]Boston Univ., Boston, MA; [2]Boston Univ. Sch. of Med., Boston, MA

Abstract: Distinct neocortical areas differ in regard to neuronal diversity, but how this diversity is achieved during development is still poorly understood. Radial Glial Cells (RGCs), the neural

stem cells of the developing cortex, divide to generate neurons directly or give rise to intermediate progenitor cells (IPCs), which also generate new neurons. Our overall hypothesis is that distinct streams of neurogenesis underlie neuronal diversity in the neocortex. Indeed our group recently reported that neurons exhibit distinct morphological and electrophysiological properties in layer 3 of the mouse frontal cortex depending on their precursor type of origin. Using a novel genetic fate-mapping technique we labeled Tbr2-expressing progenitors and their neuronal progeny as well as those derived from precursor that do not express Tbr2 with red and green fluorescent proteins respectively. Using this strategy, we fate-mapped neurons in layer 4 of the barrel cortex via in utero electroporation at E13.5. At postnatal day 21, we used whole-cell patch clamp recordings to assess the electrophysiological properties of these neurons and then subsequently assessed their morphology using high-resolution confocal microscopy. We found that both Tbr2 and non-Tbr2 progenitors generated both pyramidal and stellate neurons. Our preliminary results also suggest that non-Tbr2 neurons have higher rheobase and lower firing rates than Tbr2 neurons, consistent with our previous findings in the frontal cortex.
In addition, we studied whether neurons derived from distinct precursor streams are distributed differentially across the depth and distinct compartments of the barrel field. Interestingly, non-Tbr2 neurons were localized at more superficial (closer to the pia) levels within layer 4 than Tbr2 neurons. Further, the non- Tbr2 neurons were preferentially localized in the center of the barrel, the compartment that receives input from the barrel field, while the Tbr2-derived neurons were more frequently located in the septum.
This study contributes to a better understanding of how different streams of neurogenesis contribute to adult neuronal diversity and provides specific insight on the development of the barrel cortex, a commonly used system to model neocortical microcircuitry.

Disclosures: **T. Guillamon-Vivancos:** None. **M. Medalla:** None. **W.A. Tyler:** None. **T.F. Haydar:** None. **J.I. Luebke:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.15/C6

Topic: B.12. Glial Mechanisms

Support: CIHR Grant 410000180 - CARLEN P - CIHR - MOP 119603

Title: Connexin 43 blockade increases extracellular potassium without causing seizures in the mouse neocortex

Authors: *P. BAZZIGALUPPI[1], B. STEFANOVIC[2], I. WEISSPAPIR[1], L. LEYBAERT[3], P. CARLEN[1];
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Abstract: Extracellular potassium concentration, $[K^+]_o$, is a major determinant of neuronal excitability. In the healthy brain $[K^+]_o$, levels are tightly controlled. During seizures, $[K^+]_o$ increases up to 15mM and is thought to cause seizures due to its depolarizing effect. Although astrocytes have been suggested to play a key role in the redistribution of excess K^+ through Connexin43 (Cx43)-based Gap Junctional (GJ) coupling, the relation between this highly dynamic regulatory process and seizure generation is still unknown. Here we examined the role of astrocytic Cx43-Gjs and hemi-channels in $[K^+]_o$ regulation *in vivo*, contrasting the effects of selective blockers *vs.* broad-spectrum neuronal and astrocytic GJ blocker, Carbenoxolone (CBX). $[K^+]_o$ was measured by a K^+-sensitive microelectrode and neuronal excitability estimated by local field potential (LFP) responses to forepaw stimulation and changes in the power of resting state neuronal activity. Starting at the control $[K^+]_o$ level of 1.61 ± 0.3 mM, cortical microinjection of CBX increased $[K^+]_o$ to 11 ± 3 mM, whereas the selective Cx43-Gjs GAP27 increased it from 1.9 ± 0.7 to 9 ± 1 mM. At these elevated $[K^+]_o$ levels, no seizure activity was observed. Cx43 hemi-channel blockage by the selective peptide TAT-GAP19 increased $[K^+]_o$ by only ~1 mM. Microinjection of 4-AP increased $[K^+]_o$ to levels comparable to those achieved with CBX or GAP27 administration and induced spontaneous and recurring seizures. On the other hand, external application of increased $[K^+]_o$ to over 12 mM did not trigger seizures. These findings are the first *in vivo* demonstration that astrocytic GJs are major determinants of $[K^+]_o$, but that their blockade alone does not trigger seizures in the neocortex.

Disclosures: P. Bazzigaluppi: None. **B. Stefanovic:** None. **I. Weisspapir:** None. **L. Leybaert:** None. **P. Carlen:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.16/C7

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS075393

Title: Single-cell analyses reveal neural progenitor fate specification during cerebral cortex development

Authors: *J. LIU[1], X. WU[2], L. YANG[2], C. BUN[1], Q. LU[1];
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Abstract: During cerebral cortex development, neural stem cells/progenitors first expand the progenitor cell pool, and then undergo differentiation, generating neurons and glia sequentially. How such developmental transitions of neural progenitor cells are regulated is not well understood. One major obstacle is the high heterogeneity of neural progenitors, such as in the developing cerebral cortex of mouse, containing radial glia cells (RGCs), intermediate progenitor cells (IPCs) or basal progenitors, astrocyte progenitors and oligodendrocyte progenitors, and etc. These progenitors coexist and were surrounded by numerous new-born or mature neurons, making study on progenitor fate transition very difficult. In the current study, we combined dual-reporter neural progenitor sorting with single-cell transcriptome deep sequencing (RNA-seq). From 3 developmental stages, we acquired the global transcriptomes of 107 purified neural progenitors: 36 at starting of neurogenesis (embryonic day E12.5); 36 at peak of neurogenesis (E15.5); and 35 at starting of gliogenesis (E18.5). At the same time, we captured and sequenced 20 IPCs at E15.5 through Tbr2 (Eomes)-GFP/Dcx-mRFP sorting. Unsupervised hierarchical clustering analyses for all the 127 cells classified them into different groups, which can be recognized by cell-type specific markers. At E12.5, 75% of cells were RGCs in cell cycle, and others were new-born and maturing IPCs. At E15.5, 7 out of 36 cells were cycling RGCs, while a big group of neurogenic progenitors and IPCs account for the majority. At E18.5, isolated progenitor cell population consisted with RGCs, IPCs, astrocyte progenitors, as well as interneuron progenitors. Surprisingly, the group of neurogenic progenitors at E15.5 was more closely related to the group of astrocyte progenitors at E18.5 than to new-born or maturing IPCs, based on clustering analyses. Furthermore, principle component analysis (PCA) revealed two distinct differentiation paths of RGCs: one path reflecting neuronal differentiation of RGCs via IPCs, and the other path delineating commitment of RGCs for becoming neurogenic or gliogenic (astrocytic) progenitors. Our data thus indicated there are two types of RGCs, each of which appeared to follow a distinct progressive restriction scheme of fate specification during cortical development. We will further present our most recent findings with this respect.

Disclosures: **J. Liu:** None. **X. Wu:** None. **L. Yang:** None. **C. Bun:** None. **Q. Lu:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.17/C8

Topic: A.01. Neurogenesis and Gliogenesis

Support: Leffler Grant. Department of Neurobiology. Harvard Medical School.

Hearst Grant. Department of Neurobiology. Harvard Medical School.

Title: Prdm16 transcriptional activity in radial glial cells controls cortical neurogenesis

Authors: *J. BAIZABAL CARBALLO, M. TURRERO, N. GÓMEZ, O. OLUKOYA, C. HARWELL;
Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Radial glial cells (RGCs) are the origin of cell diversity in the mature cerebral cortex. RGCs establish the organization of different cortical layers by progressively generating specific subtypes of excitatory neurons. At the onset of neurogenesis, early RGCs divide asymmetrically to produce neurons directly. As neurogenesis continues, RGCs transition to give rise to intermediate progenitors, which divide symmetrically to produce pairs of neurons through a process of indirect neurogenesis. At present, the molecular mechanisms whereby specific transcriptional programs control the pattern of neurogenesis by cortical RGCs are not well understood. The family of PRDM (Positive Regulatory domain-containing) genes has important functions in controlling stem cell renewal and differentiation in multiple tissues. In particular, Prdm16 is part of the evolutionary conserved gene network that is expressed in both mouse and human RGCs. Interestingly, mutations mapping within Prdm16 genomic region in human chromosome 1 are linked to abnormal development of the cerebral cortex and intellectual disability. Furthermore, previous evidence has suggested a role of Prdm16 in progenitor survival and proliferation in the mouse brain. Yet, the gross brain defects and perinatal lethality observed in Prdm16 loss of function mice has precluded a more detailed analysis. Here, we generated cerebral cortex-specific mutant mice to investigate the role of Prdm16 in cortical development. Absence of Prdm16 during embryonic neurogenesis increased cell cycle exit of RGCs. This process resulted in a significant reduction of proliferating intermediate neuronal progenitors at the subventricular zone of mutant cortices. Accordingly, quantification of the size of clonal radial units using in vivo retroviral labeling demonstrated that Prdm16 mutant RGCs produce smaller clones in comparison to controls. Our data suggest that Prdm16 maintains the undifferentiated state by inhibiting direct neurogenesis, favoring the production of intermediate progenitors and indirect neurogenesis. Currently, we are investigating the molecular mechanisms by which Prdm16 transcriptional program in RGCs controls neurogenesis in the cerebral cortex.

Disclosures: J. Baizabal Carballo: None. **M. Turrero:** None. **N. Gómez:** None. **O. Olukoya:** None. **C. Harwell:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.18/C9

Topic: A.01. Neurogenesis and Gliogenesis

Support: Polish Ministry of Science Grant Mobility Plus DPN/MOB109/II/2012

Center for Cancer Research, National Cancer Institute, NIH

Title: HMGNs affect alternative isoform regulation of mRNA in prefrontal cortex, hippocampus and embryonic stem cells.

Authors: *P. LISOWSKI[1], S. ZHANG[2], T. DENG[2], T. FURUSAWA[2], M. BUSTIN[2];
[1]iPS Cell Based Dis. Modeling Group, Max-Delbrück-Center For Mol. Med. (MDC), Berlin, Germany; [2]Protein Section, Lab. of Metabolism, Ctr. for Cancer Research, Natl. Cancer Institute, Natl. Inst. of Hlth., Bethesda, MD

Abstract: High mobility group N (HMGN) family members are nucleosome-binding proteins that affect chromatin structure. HMGN variants are expressed in most brain regions; however, their effect on the fidelity of cellular transcription profile and on neurological functions is not known. Here, we study the role of HMGN family in the modulation of transcriptome in hippocampus, prefrontal cortex, embryonic stem cells (ESCs), mouse embryonic fibroblasts (MEFs), and B cells of genetically altered mice and cell lines that either lack, or overexpress functional HMGN variants. Transcription profiles of wild-type, HMGN1/HMGN2 double knockout, and HMGN1 overexpressing mice and cell lines were analyzed by paired-end, RNA-seq using Illumina GAIIx. The sequence reads were analyzed with Cufflinks/Cuffdiff followed by Mixture of Isoforms (MISO) - a probabilistic framework towards detection of alternatively spliced genes, differentially regulated isoforms and exons across samples. To discover alternative isoform regulation of mRNA we estimated differential transcript usage (DTU), differential transcript expression (DTE), alternative 3 and 5' splice sites (A3SS, A5SS) usage, mutually excluded exons (MXE), retained introns (RI), and skipped exons (SE). We identified that the loss or overexpression of HMGNs in adult brain and ESCs result in alternative 3' and/or 5' splice sites usage of genes involved in transcription regulation such as tRNA methyltransferase 1 (TRMT1) in prefrontal cortex or HOXA1 encoding DNA-binding transcription factor regulating gene expression patterns responsible for morphogenesis, differentiation, as well as placement of hindbrain segments in the proper location along the anterior-posterior axis during development. Thus, epigenetic factors such as HMGNs could affect the proper functioning of central nervous system by modulating the transcriptional profiles in stem cells as well as in cortical and hippocampal neurons. This study posits a role of HMGNs

in nervous system development, and provides a basis for further hypotheses on the role of HMGNs in alternative isoforms regulation of mRNA.

Disclosures: **P. Lisowski:** None. **S. Zhang:** None. **T. Deng:** None. **T. Furusawa:** None. **M. Bustin:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.19/C10

Topic: A.01. Neurogenesis and Gliogenesis

Support: Lejeune Foundation Grant #1209

Title: Mechanisms of cell fate refinement in the embryonic cortex are deregulated by lack of Mecp2

Authors: *C. COBOLLI GIGLI[1], L. SCARAMUZZA[1], C. KILSTRUP-NIELSEN[2], N. LANDSBERGER[1,3], F. BEDOGNI[1];
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Abstract: During development, neurons progressively restrict their fate repressing the expression of specific genes while differentiating. This process vastly relies on transcription and is therefore subject to epigenetic control. The Methyl-CpG binding protein 2 (MeCP2) is an epigenetic factor that, binding methylated DNA, participates to chromatin folding and transcriptional regulation. Mutations of *MECP2* leads to Rett syndrome (RTT), a devastating condition whose pathogenetic mechanisms are not yet completely understood but most likely originate from developmental derangements. We hypothesised that lack of this epigenetic factor may alter proper cellular maturation due to misinterpretation of the DNA methylation signature characterizing each cell type throughout its development. To evaluate this hypothesis, we analyzed the developing *Mecp2* null cerebral cortex. Interestingly, at the embryonic day 15.5 (E15.5) the transcriptional identity of proliferating cortical progenitors is increased in null cortices compared to wt. Since proper cortical development is ensured by a tightly regulated progression through cell cycle phases, we tested whether dynamics of neurogenesis were deregulated in null samples. Interestingly, rather than altering cell cycle progression, lack of Mecp2 affects the expression of markers that are typical of cells transitioning from one stage to the next one.This suggests that null cells, switching from one cellular type to the next one, retain parts of the previous identity, possibly due to the lack of control over transcription normally

exerted by Mecp2. Given proper proliferation dynamics of cortical progenitors are crucial for the subsequent development of newborn neurons, it is possible to infer that the known maturation delay affecting the developing Mecp2 null cortex could be generated by derangements in the control of cell fate refinement.

Disclosures: C. Cobolli Gigli: None. **L. Scaramuzza:** None. **C. Kilstrup-Nielsen:** None. **N. Landsberger:** None. **F. Bedogni:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.20/C11

Topic: A.01. Neurogenesis and Gliogenesis

Title: The role of Fezf2 in regulating postnatal ventricular zone stem cell fate

Authors: *A. A. AKHTAR, M. DUTRA-CLARKE, G. KIM, R. LEVY, S. SAVINOFF, M. DANIELPOUR, J. BREUNIG;
RMI, Cedars Sinai Med. Ctr. Regenerative Med. Inst., West Hollywood, CA

Abstract: The transcription factor Fezf2 has been identified as a critical determinant of layer 5 corticofugal projection neurons. This class of neurons includes corticospinal motor neurons that are lost in degenerative motor neuron disorders such as amyotrophic lateral sclerosis (ALS). Previous work has explored the ability of Fezf2 to reprogram cells to a corticofugal phenotype within a very spatial and temporal window of early development. Interestingly, Fezf2 is expressed by a large majority of dorsal ventricular zone (VZ) stem and progenitor cells. We have developed an electroporation-based method for stably expressing Fezf2 in neural stem cells lining the lateral ventricle. Increasing expression of Fezf2 in these cells reduces astrogliogenesis and olfactory bulb (OB) neurogenesis. To avoid acute OB neurogenesis alterations, we developed an inducible and reversible, 3rd generation, doxycycline(Dox)-regulated genetic system for expressing Fezf2. When our expression vector is induced by Dox in cultures of mouse neural stem cells or astrocytes, Fezf2 causes morphological conversion into neuron-like cells despite the presence of growth factors and serum. When Fezf2 expression is induced postnatally in the olfactory bulb, we see changes in nuclear size, increased ER81 expression, and evidence of ectopic axonal growth from the olfactory bulb. Using this new technology, we are exploring the ability of Fezf2 to reprogram heterogeneous populations of stem, progenitor and terminally differentiated cells to corticofugal subtypes in postnatal and adult mice.

Disclosures: **A.A. Akhtar:** None. **M. Dutra-Clarke:** None. **G. Kim:** None. **R. Levy:** None. **S. Savinoff:** None. **M. Danielpour:** None. **J. Breunig:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

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Program#/Poster#: 492.21/C12

Topic: A.01. Neurogenesis and Gliogenesis

Support: F30 NRSA fellowship PA-14-150-NIMH

Human Frontiers Science Programme Postdoctoral Fellowship LT000075/2014-L

NIH grant 1RO1MH101268-01

Title: Birth time-dependent specification of pyramidal neuron laminar and projection types through intermediate progenitors

Authors: *J. M. LEVINE[1,2], D. HUILGOL[1], M. HE[3], Z. J. HUANG[1];
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Abstract: Pyramidal neurons (PyNs) comprise the majority of cortical neurons and underlie nearly all aspects of cognitive operations. The progenitor cells that give rise to neocortical PyNs mainly include radial glial cells (RGCs) and intermediate progenitor cells (IPCs) located in the embryonic cerebral ventricle wall. RGCs divide asymmetrically to generate neurons either directly or indirectly through IPCs, which divide symmetrically to produce pairs of PyNs. It remains unclear how progenitor types (e.g. RGCs, IPCs), their lineage progression, and timing of neurogenesis contribute to the specification of diverse PyN subtypes defined by axon projection, connectivity, and physiology. In particular, the role of IPCs in the generation of PyNs is poorly understood. The T-domain transcription factor (Tbr2) is specifically expressed in cortical IPCs. We have generated an inducible *Tbr2-CreER* mouse driver, which allows comprehensive lineage tracing from IPCs and have developed a novel genetic method to fate-map neurons according to their lineage and precise birth time. We have used the *Tbr2-CreER* driver and Cre-dependent reporter mice to fate map IPCs throughout embryogenesis. In addition to assessing the laminar position of PyN subtypes, axon projections are analyzed with a novel method utilizing viral labeling of fate-mapped PyNs. Using retrograde virus along with our genetic driver and reporter lines allows us to restrict cell labeling by progenitor type, birth date, and projection target. With this method, we can further elucidate the PyN subtypes born from IPCs throughout neurogenesis,

using axon projection as more descriptive definition of cell type than laminar location. Fate mapping experiments revealed that IPCs sequentially gave rise to PyNs with distinct laminar patterns spanning multiple nonconsecutive layers with only a trend towards an inside-out sequence. This suggests that IPCs do not generate PyNs in a strictly inside-out manner. Rather, temporal cohorts of multiple fate-restricted IPCs simultaneously, as well as sequentially, generate PyN subtypes defined by their axon projection and laminar location. Ongoing experiments include an investigation of the PyNs that are generated by direct neurogenesis from RGCs, in contrast to those that are generated by IPCs, and the development of a novel genetic birth-dating strategy utilizing the Tis21 gene that is expressed in progenitors as they undergo neurogenic division. These findings begin to link progenitor type and their time of neurogenesis to the specification of PyN subtypes.

Disclosures: **J.M. Levine:** None. **D. Huilgol:** None. **M. He:** None. **Z.J. Huang:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Human Frontiers Science Programme Postdoctoral Fellowship LT000075/2014-L to DH

F30 NRSA fellowship PA-14-150-NIMH to JML

NIH grant 1RO1MH101268-01 to ZJH

Title: Role of intermediate progenitors in the specification of cortical pyramidal neurons

Authors: *D. HUILGOL[1], J. M. LEVINE[1], M. HE[1,2], P. WU[1], Z. J. HUANG[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Inst. of Brain Sci., Fudan Univ., Shanghai, China

Abstract: The neocortex covers the anterior-most portion of the mammalian brain and is involved in a range of cognitive functions. It consists of multiple layers with distinct neuronal types, of which pyramidal or projection neurons (PyNs) constitute ~80% of cortical neurons. PyNs mediate multiple information processing streams and all the major cortical output channels, and are diverse in terms of their laminar location, gene expression, connectivity and physiology. However, the developmental origins of this diversity are poorly understood. PyNs arise from mainly two types of progenitors: the radial glial cells (RGCs) and the intermediate

progenitor cells (IPCs). It is unclear how these progenitors, their lineage progression and timing of neurogenesis contribute to specification of diverse PyN identities, for example defined by their projection targets. In particular, the role of IPCs is not understood. We have generated an inducible knock-in mouse driver for Tbr2 (*Tbr2*-creER), a T-domain transcription factor which is specifically expressed by the IPCs and is necessary for their identity. This allowed us to perform comprehensive lineage tracing for PyNs born from IPCs. Cortical neurogenesis is known to follow an inside-out sequence of layer formation, such that neuronal birth timing relates to its laminar identity. Fate mapping at different embryonic ages using a reporter bred with *Tbr2*-creER line reveals that PyN generation does not follow the expected inside-out pattern of sequential laminar generation. We observed that PyNs produced at specific time points span multiple non-consecutive layers suggesting the simultaneous presence of multiple fate-restricted IPCs at these ages. Analysis of the progenitors in terms of their location in the germinal zone, their morphology and markers revealed that they are indeed IPCs. We hypothesize that PyNs are born based on their projection targets, which follow a broad but not a strict inside-out sequence of birth order. To achieve high resolution fate mapping, we have performed clonal analysis from Tbr2(+) progenitors using MADM (Mosaic Analysis with Double Markers) and Brainbow mice. Pairs of PyNs produced at different developmental time points are similar in morphology and laminar positions. We are currently utilizing different clearing techniques to analyze and compare the projections of PyNs born from a single IPC. Ongoing experiments also include axon tracing by using viral labeling methods to characterize PyN subtypes beyond their laminar positions.

Disclosures: D. Huilgol: None. **J.M. Levine:** None. **M. He:** None. **P. Wu:** None. **Z.J. Huang:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.23/C14

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS084398

Title: Endogenous and hemorrhage-derived lysophosphatidic acid (LPA) signaling alters mitotic spindle orientation and subsequent cell fates of neuroprogenitor cells in the mammalian cerebral cortex

Authors: *Y. C. YUNG, W. MCDONALD, J. CHUN;
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Abstract: Mitotic spindle and cleavage plane orientation are critical factors influencing symmetric vs asymmetric cell division during cortical development. Their intracellular molecular pathways and associated fate determinants have been progressively characterized. However, soluble, extracellular signals affecting cleavage plane are now being revealed, including recent studies in semaphorin signaling. Here we report that lysophosphatidic acid (LPA), an extracellular lipid signaling molecule present in cerebrospinal fluid, shifts cleavage planes through different G protein-coupled LPA receptor subtypes and downstream pathways. Dual genetic removal of LPA_1 and LPA_2 produced changes in cleavage orientation. In addition, perturbation of endogenous LPA levels via *ex vivo* cortical hemisphere cultures or *in vivo* fetal cerebral cortical injections resulted in altered apical adherens junctions, cell polarity, apico-basal mitotic spindle orientation, and subsequent altered neural fates compared with controls. Exposure to either blood plasma or serum - known significant sources of LPA - consistent with hemorrhage also produced similar changes. Genetic removal of both LPA_1 and LPA_2 receptors abrogated LPA, plasma, or serum-induced changes, yet also resulted in unstimulated cleavage plane orientation alterations comparable to wild-type controls, indicating normal influences on cleavage plane through endogenous LPA actions on its receptors. These data identify LPA as a soluble, extracellular signal acting through two cognate LPA receptors to influence neuroprogenitor cell fate under basal development and neuropathological conditions that elevate LPA, including hemorrhage and hypoxia. These pathological stimuli alter brain development which contribute to diseases such as hydrocephalus and likely other hemorrhage-related neurodevelopmental disorders.

Disclosures: **Y.C. Yung:** None. **W. McDonald:** None. **J. Chun:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

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KAKENHI 15H01293

KAKENHI 15K09723

KAKENHI 26430075

KAKENHI 16H01343

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KAKENHI 15K02355

Title: Morphological analyses of radial glial cells in the developing mouse neocortex

Authors: ***M. SHIN**[1], A. KITAZAWA[2], Y. MATSUNAGA[1], K. HAYASHI[1], K.-I. KUBO[1], K. NAKAJIMA[1];
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Abstract: During mammalian neocortical development, excitatory neurons are generated directly or indirectly from specialized progenitors called radial glial cells (RGCs), located in the ventricular zone (VZ). Newly born neurons migrate toward the outermost superficial region of the cortical plate by passing through their predecessors. In the course of this 'inside-out' manner of neuronal alignment, radial fibers extended from RGCs to the pial surface play as a scaffold for migrating neurons, resulting in their columnar configuration which might contribute to the development of functional units in the future. To establish neuronal arrangement successfully, radial fibers are required to maintain their long thin structure properly for providing for migrating neurons.Reeling glycoprotein, which is secreted by Cajal-Retzius cells in the marginal zone, is known to be one of the key molecules for establishing the 'inside-out' neuronal layers, since Reelin-deficient mice, called *reeler*, show roughly inverted neuronal layers. Although much about the molecular and functional basis of Reelin singling has been elucidated in the context of neuronal migration, little is known about the effects of Reelin on the RGCs. Several lines of evidence in mammalian neocortical development have found that Reelin can affect morphology of radial fibers, however, the biological role of Reelin on RGCs still remains to be elucidated.To investigate whether structure of RGCs is disrupted in the *reeler* mutant mice, we electroplated a GFP-expression plasmid into E14-16 heterozygous and homozygous *reeler* embryonic cortices to visualize radial fibers with GFP. To examine the fiber morphology in detail, we quantitively analysed the fibers after imaging at high resolution. Fibers in the *reeler* mutant had occupied approximately 6μm more in width when transversing the entire cerebral wall, compared to those in the control mice. 3D imaging was also carried out to reveal differences of radial fibers between normal and *reeler* mutant mice.

Disclosures: **M. Shin:** None. **A. Kitazawa:** None. **Y. Matsunaga:** None. **K. Hayashi:** None. **K. Kubo:** None. **K. Nakajima:** None.

Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 492.25/C16

Topic: A.01. Neurogenesis and Gliogenesis

Title: Classification of adult human cortical cell types using single nucleus rna-seq

Authors: *R. D. HODGE[1], T. BAKKEN[1], J. A. MILLER[1], M. NOVOTNY[2], S. I. SHEHATA[1], B. D. AEVERMANN[2], P. VENEPALLY[3], K. SMITH[1], D. N. TRAN[2], J. MCCORRISON[2], F. DIEZ FUERTES[2], S. M. SUNKIN[1], R. H. SCHEUERMANN[2,4], R. S. LASKEN[2], E. S. LEIN[1]; [1]Allen Inst. For Brain Sci., Seattle, WA; [2]J. Craig Venter Inst., La Jolla, CA; [3]J. Craig Venter Inst., Rockville, MD; [4]Pathology, UCSD, San Diego, CA

Abstract: The human cerebral cortex is a remarkably complex structure comprised of billions of cells. However, despite many years of research focused on classifying cell types using morphological, electrophysiological, and gene expression data, a thorough understanding of the complete range of cell types that populate the human cerebral cortex is lacking. Here we investigated the potential of using RNA-seq on single nuclei isolated from adult human neocortex to generate transcriptomic signatures and derive cell type classifications. Single nucleus RNA-seq was used to capture the transcriptomes of >1000 individual nuclei isolated from adult human postmortem brain samples. We focused our analyses on two specific layers within distinct cortical regions, layer 1 of middle temporal gyrus and layer 5 of frontoinsular cortex, in order to access a diversity of inhibitory and excitatory neuron types of high biological interest. Layer 1 of middle temporal gyrus contains several anatomically and physiologically distinct classes of GABAergic interneurons, including neurogliaform cells. Layer 5 of frontoinsular cortex contains a variety of excitatory and inhibitory cell types, as well as a high concentration of von Economo neurons, a type of projection neuron described in relatively few species and particularly enriched in select regions of human cortex. Neuronal nuclei were specifically labeled using an antibody against NeuN, and individual neuronal (NeuN-positive) and non-neuronal (NeuN-negative) nuclei were captured using fluorescence-activated cell sorting. cDNA libraries from individual nuclei were constructed using a modified Smart-seq2 protocol and sequenced on a HiSeq instrument at a depth of >1 million reads/sample. Our results indicate that single nucleus RNA-seq successfully discriminates major cell types in the adult cortex, including excitatory and inhibitory neuronal types and glial cell types. Furthermore, iterative clustering revealed multiple subtypes of inhibitory and excitatory neurons in our target regions of interest. These results demonstrate the utility of single nucleus RNA-seq for characterizing the diversity of cell types in the adult human neocortex and provide insight into the biochemical and functional differences between cell types.

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Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: EMBO long-term fellowship ALTF 861-2013

DFG Grant SFB 655, A2

ERC Grant 250197

Title: CRISPR/Cas9-induced disruption of gene expression in mouse embryonic brain and single neural stem cells *In vivo*

Authors: ***N. KALEBIC**, E. TAVERNA, S. TAVANO, F. K. WONG, D. SUCHOLD, S. WINKLER, M. SAROV, W. B. HUTTNER;
MPI-CBG, Dresden, Germany

Abstract: *In vivo* manipulation of gene expression is a major way of studying the function of individual genes in their physiological context. Here we applied the CRISPR/Cas9 system to disrupt gene expression in neural stem cells in the developing mammalian brain *in vivo*.
As a proof-of-principle we first targeted GFP in a knock-in mouse line, where GFP is expressed in a subset of neural progenitor cells during embryonic cortical development. We electroporated embryonic brains *in utero* with a single plasmid encoding Cas9, guide RNA (gRNA) and a fluorescent marker, and obtained nearly complete (90%) disruption of GFP expression two days after targeting. Considering that changes in cell fate during cortical development typically occur within a single progenitor cell cycle, electroporation of the Cas9/gRNA plasmid has a potential drawback that any genome editing can only occur after Cas9 and the gRNA have been produced. To overcome this limitation, we directly electroporated recombinant Cas9/gRNA complex and achieved near-maximal efficiency of disruption of GFP expression already 24 hours after electroporation. Strong disruption of GFP expression within a single cell cycle was detected after microinjecting the Cas9/gRNA complex into single neural stem cells in organotypic slice cultures, an approach that enabled us to trace the fate of individual cells upon genome editing.

Finally, we applied these approaches to disrupt the expression of *Eomes*, a gene fundamental for neocortical neurogenesis. This resulted in a reduction in basal progenitors, a major pool of neural progenitors in the developing cortex, and an increase in neuronal differentiation. Sequencing analysis revealed no detectable indels in the four major off-target sites, suggesting that the observed neurodevelopmental phenotype was indeed caused by selective disruption of the *Eomes* locus.
Thus, we successfully applied the CRISPR/Cas9 system to achieve rapid, efficient and enduring disruption of gene expression followed by fate tracing of immediate daughter cells during mammalian brain development *in vivo*.

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Poster

492. Cerebral Cortex: Fate Specification and Neuronal Differentiation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Wellcome Trust (Grants 094832/Z/10/Z)

BBSRC (BB/M00693X/1)

Title: Roles of differential sulfation in Fgf8/Erk signaling during mouse forebrain development.

Authors: *W. CHAN, D. PRICE, T. PRATT;
Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Heparan sulfate proteoglycans (HSPGs) are cell surface/secreted molecules expressed by all cells. HSPGs consist of linear heparan sulfate (HS) carbohydrate side-chains attached to a core protein and are involved in regulating key signalling pathways in the developing mammalian brain via sugar-protein interactions. HS has an enormous variety of structures due to postsynthetic modification. HS hypothesis states that the specificity for the interaction between the HSPGs and particular signalling pathways is encoded by differential HS sulfation. Hs2st and Hs6st1 are enzymes involved in generating different HS structures by sulfating the 2-O or 6-O carbon molecule of the sugar backbone respectively. Loss of either Hs2st or Hs6st1 function has profound, but distinct, consequences for forebrain development confirming their importance. The distinct effects differential sulfation have on forebrain development suggests that specific HS structures drive distinct developmental programmes via the regulation of signaling pathways

supporting the HS code hypothesis. Fibroblast growth factor (Fgf) is a family of signaling molecules crucial for forebrain development. Fgf8 is a secreted morphogen where it functions to pattern the forebrain via regulated gradient formation. Fgf8 protein levels and the interpretation of the Fgf8 protein gradient are important for Fgf8 signaling. HS has been previously shown to be involved in these processes however, the role differential sulfation plays in these processes and particularly, the molecular mechanism(s) behind this has not been clearly resolved. Fgf8 signaling has been widely studied and its signaling mechanism well characterised. Yet, the conventional model of its signaling mechanism could not fully explain the observations preliminary to this work where *Hs2st* and *Hs6st1* mutants have disrupted Fgf8/Erk signaling, presenting a gap in our current understanding of Fgf8/Erk signaling. We developed an *ex vivo* assay to probe the formation of Fgf8 gradient over time and the regulation of Fgf8 gradient formation and signaling by differential HS sulfation. Our data shows that specific HS sulfation regulates Fgf8 distribution and its interpretation in distinct ways supporting the HS code hypothesis. Our *ex vivo* data allowed us to formulate predictions about the regulation of the Fgf8 gradient and its interpretation *in vivo*. Testing these predictions *in vivo* enabled us to model the regulation of the Fgf8 gradient and its interpretation by differential sulfation during forebrain development. This provides us with further insight into the role of HS in the complex but precise regulation of mouse forebrain development.

Disclosures: **W. Chan:** None. **D. Price:** None. **T. Pratt:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

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Program#/Poster#: 493.01/C19

Topic: A.01. Neurogenesis and Gliogenesis

Title: The zinc finger transcription factor sp8 and sp9 coordinately regulate the generation of striatopallidal projection neurons

Authors: ***Z. XU**[1], Q. LIANG[1], L. AN[1], Y. YOU[1], Z. ZHANG[1], S. WEI[1], Z. LI[1], C. WANG[2], Z. YANG[1];
[1]Inst. of Brain Sci. and State Key Lab., Fudan Univ., Shanghai City, China; [2]Dept. of Neurology, Affiliated Hosp. of Hebei Univ. of Engin., Handan, China

Abstract: Striatal medium-sized spiny neurons (MSNs), composed of striatonigral and striatopallidal neurons, are derived from the lateral ganglionic eminence (LGE). We previously have shown that, in Sp9 mutant mice, LGE striatopallidal MSN progenitors have reduced proliferation. Moreover, most striatopallidal MSN progenitor cells fail to differentiate into

mature striatopallidal neurons, and die cell-autonomously through Bax-dependent apoptosis. On the other hand, development of striatonigral MSNs is largely unaffected. In the present study, we found that in the striatum of Sp8 and Sp9 double conditional mutant mice, none of striatopallidal MSNs was produced. This was due to cell cycle arrest of striatopallidal MSN progenitors, which further induced these progenitors undergoing apoptosis in the LGE SVZ, but not in the striatum. Once again, development of striatonigral neurons appears normal. Diverse molecular mechanisms underlying these phonotypes are currently under study.

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Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

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Topic: A.01. Neurogenesis and Gliogenesis

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Boehringer Ingelheim Fonds to WMK

Title: Nkx2.9 programs mesodiencephalic dopaminergic neurons early in development

Authors: ***W. M. KOUWENHOVEN**[1,2], L. VON OERTHEL[1], C. WAGEMANS[1], I. HOUWERS[1], J. TIAN[2], M. GRUPPILO[2], J. LOCKER[2], M. P. SMIDT[1];
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Abstract: Nkx2.9 (or Nkx2.8 in most other species, except for mouse) is a member of the NK homeobox family and resembles Nkx2.2 based on homology and on its expression pattern in the central nervous system. However, while Nkx2.2 is required for the development of serotonergic neurons, the role of Nkx2.9 in the mid-hindbrain region is still ill-defined. In a recent paper from our group we investigated the role of homeobox transcription factor Engrailed 1 (En1) in mid-hindbrain development, and designated Nkx2.9 as a possible transcriptional target of En1 (Veenvliet et al., 2013). In the current work we elaborate on this finding by determining whether mesodiencephalic dopaminergic (mdDA) neurons require Nkx2.9 during their molecular development. Here, we describe for the first time that Nkx2.9 contributes significantly to the molecular programming of mdDA neurons. Lineage-tracing reveals that a large majority of mdDA neurons encounter Nkx2.9 expression during early embryonic development. Furthermore,

we demonstrate using next generation RNA-Seq and *in situ* hybridization on Nkx2.9-ablated animals, that Nkx2.9 influences the expression of the dopamine transporter (Dat) in mdDA neurons.

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Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.03/C21

Topic: A.01. Neurogenesis and Gliogenesis

Support: Smoking Research Foundation

Title: α7 nicotinic acetylcholine receptors mediate nicotine-induced neurite outgrowth of rat superior cervical ganglia cells

Authors: *S. TAKATORI[1], H. HINO[2], F. TAKAYAMA[2], Y. KITAMURA[3], T. SENDO[3], H. KAWASAKI[4];
[1]Matsuyama Univ., Matsuyama, Japan; [2]Dept. of Clin. Pharmaceut. Sciences, Grad. Sch. of Medicine, Dent. and Pharmaceut. Sciences, Okayama Univ., Okayama, Japan; [3]Dept. of Pharmacy, Okayama Univ. Hosp., Okayama, Japan; [4]Dept. of Clin. Pharmacy, Col. of Pharmaceut. Sciences, Matsuyama Univ., Matsuyama, Japan

Abstract: ***Background****:* The previous *in vivo* study demonstrated that nicotine facilitates only reinnervation of sympathetic adrenergic nerves injured by topical phenol-application in the rat mesenteric artery, and markedly increased levels of nerve growth factor (NGF) contents and the expression of NGF receptor TrkA in superior cervical ganglia (SCG), which were inhibited by the pretreatment of nicotinic acetylcholine receptor (nAChR) antagonist hexamethonium (Takatori S *et al*., Eur J Pharmacol, 748: 1-9, 2015.). To clarify possible mechanisms, the present study was further investigated the effect of nicotine on neurite outgrowth of primary cultured-SCG cells *in vitro*. ***Methods****:* SCG cells isolated from Wistar neonate rats were primarily cultured for 5 days. Numbers of neurite outgrowth from cell body were measured in the presence of nicotine (10-3000 μM), acetylcholine (ACh) (10-3000 μM), or NGF (10-100 ng/mL). Hexamethonium (100 μM) or α-bungarotoxin (100 nM) was incubated with nicotine for 5 days. ***Results****:* Nicotine (10-1000 μM) concentration-dependently increased neurite outgrowth numbers from tyrosine hydroxylase-immunopositive SCG cells, while high concentration of 3000 μM did not increase the numbers. The nicotine-induced increase in neurite numbers was dependent on

the exposure time (8-24 hr/day) of nicotine. Combined incubation with nicotine and hexamethonium or α-bungarotoxin did not increase neurite outgrowth of SCG cells. ACh at 10-1000 μM had no effect on the neurite numbers, but high concentration of 3000 μM markedly increased them. NGF also markedly increased neurite outgrowth numbers of SCG cells. Combined incubation with nicotine (10-1000 μM) and NGF (10 ng/mL) resulted in an additive increase in NGF-induced neurite numbers. ***Conclusion****:* These results suggest that nicotine has a neurotrophic effect on sympathetic ganglia cells via activation of α7 nAChR. (This study was supported by Smoking Research Foundation).

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Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

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Title: TRPM8 as a candidate channel to regulate temperature-dependent changes in spinal neuron differentiation

Authors: *K. A. SPENCER[1,2], L. N. BORODINSKY[1,2];
[1]Shriners Hosp. For Children Northern CA, Sacramento, CA; [2]Physiol. and Membrane Biol., Univ. of California Davis, Davis, CA

Abstract: Developmental rate of ectotherms, including frogs, is dependent on the temperature of the environment. However, it is unknown how environmental temperature influences nervous

system development. Neuronal differentiation in the spinal cord is dependent on spontaneous Ca^{2+} activity. We hypothesize that factors in the environment may affect neuronal specification by modulating this spontaneous Ca^{2+} activity. In this study, we investigate the role of temperature in spinal neuron differentiation in *Xenopus laevis*.
Data show that growing tadpoles at cold temperature (14.5°C) results in larger animals that are about 10% longer and have an overall number of cells in the spinal cord 15 and 25% higher compared to warmer temperature-grown tadpoles (22.5 and 26.5, respectively). Strikingly, the number of motor neurons is 100% higher in cold temperature-grown tadpoles. In addition, the distance between sensory axon bundles projecting in the axial skeletal muscle is significantly longer in tadpoles grown at 14.5°C than those grown at 26.5°C. Further analysis of morphological features of both sensory and motor neurons in embryos grown at different temperatures is underway.
To investigate the mechanisms underlying these temperature-driven changes in neuron specialization, I assessed the effect of TRPM8, a cold sensitive channel, on spontaneous Ca^{2+} spike activity. I first evaluated the expression of TRPM8 by RT-PCR and immunostaining and find that TRPM8 is present in the embryonic spinal cord. Blocking TRPM8 activity with 10 μM AMTB, reverses the cold-temperature-induced increase in Ca^{2+} spike frequency in ventral spinal neurons. Additional experiments will further explore whether a TRPM8-mediated increase in Ca^{2+} spike frequency at low temperatures influences motor neuron differentiation.
Altogether these results demonstrate that temperature regulates spinal cord development in *Xenopus laevis*, and suggest this may work through TRPM8. The findings indicate that the environment intervenes in the differentiation program of developing neurons.

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Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

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Title: Roles of Tbr1 in retinal ganglion cell subtype formation

Authors: *C.-A. MAO[1,2], C. M. WHITAKER[2], P. PAN[2], T. C. BADEA[3], J. PARKER-THORNBURG[4], W. H. KLEIN[5], S. L. MILLS[2], S. C. MASSEY[2];
[1]Ophthalmology & Visual Sci., Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [2]Ophthalmology and Visual Sci., The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [3]Natl. Eye Institute, Natl. Inst. of Hlth., Bethesda, MD; [4]Genet., [5]Systems Biol., The Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Background and Objective Retinal ganglion cells (RGCs) are the only output neurons that collectively transmit visual information from the retina to different regions in the brain. In the mouse retina, some 20 to 30 subtypes of RGCs have been described based on morphology and/or function. However, the genetic regulatory mechanism controlling RGC subtype specification and differentiation remains vaguely understood, in part because of the difficulty in experimentally tracing a specific RGC subtype lineage through development. Here we describe experiments demonstrating that T-box transcription factor T-brain 1 (Tbr1) is expressed in approximately 11% of mouse RGCs. By using genetically-directed sparse labeling and dye-filling on individual RGC, we have uncovered two separate RGC subtypes expressing Tbr1. Methods To conduct sparse-labeling and dye-filling in Tbr1-expressing RGCs, we generated $Tbr1^{CreERT2/+}$:$Pou4f1^{cKO\text{-}AP}$ and $Tbr1^{CreERT2/+}$:Ai9 mouse lines, respectively. Tamoxifen was injected intraperitoneally into these mice for five consecutive days to activate Cre recombinase, and hence turn on $Pou4f1^{cKO\text{-}AP}$ or Ai9 reporter lines. We then performed flat-mount alkaline phosphatase (AP) staining on the retinas from tamoxifen-injected $Tbr1^{CreERT2/+}$: $Pou4f1^{cKO\text{-}AP}$ mice to reveal the RGC subtypes expressing Tbr1. Furthermore, we conducted neurobiotin-filling in individual Ai9/tdtomato-marked Tbr1+ RGCs in tamoxifen-injected $Tbr1^{CreERT2/+}$:Ai9 mice to systematically examine the detailed dendritic morphologies of Tbr1-expressing RGCs. Results and Conclusions With both mouse lines, we provide evidence for two morphologically distinct RGC subtypes within Tbr1+ RGCs, including the JAM-B RGC, an upward motion-detecting RGC subtype, and a RGC subtype which stratifies in the OFF layer of the inner plexiform layer. These mouse lines provide a unique opportunity to investigate the differentiation, growth, the central projection and physiology of these RGC types, and to understand the genetic regulatory mechanisms that control the development of these RGCs.

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Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

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Title: Pioneering connections from brain to retina, which might be a unique human specialisation

Authors: *I. BYSTRON[1], C. BLAKEMORE[2];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Sch. of Advanced Study, Inst. of Philosophy, London, United Kingdom

Abstract: Although the proliferative zones of retina and ventral diencephalon derive from the same region of the embryonic prosencephalon, they generate very different classes and distributions of neurons. We are interested in the relationship between cell production and migration in the human retina and neighboring diencephalon.
Human embryos from Carnegie stages (CS) 10-19 (29-44 days post-conception) were obtained according to national guidelines in Russia and from the Human Developmental Biology Resource UK. Different components of the neural stem cell niche in the diencephalon, optic stalk and presumptive retina were reconstructed by rapid, interactive, high-resolution volume rendering of multichannel 3D confocal data sets from a Zeiss LSM 710 confocal microscope. We observed a previously unreported early neuronal population with a very unusual pattern of migration. Typically, in the mammalian forebrain (including the retina), neurons born locally in the ventricular zone (VZ) of the neuroepithelium migrate radially and accumulate under the pial surface. However, the majority of first-born neurons in human embryonic hypothalamus (at CS 12) appear to migrate tangentially along the surface of the ventral diencephalon lining the third ventricle, towards the optic vesicle. By CS13-14 these precocious cells, which we call Meitar neurons, invade the neural and pigmental retina. The leading processes of Meitar neurons arrive in the dorso-central vitreal surface of the retinal epithelium at CS16, followed at CS17 by the appearance of the first locally generated ganglion cells at the same location. The leading and trailing processes of Meitar neurons, with multiple varicosities, are easily distinguishable from the smooth GAP-43-positive axons of ganglion cells. The axons of the first ganglion cells follow the processes of the pioneering Meitar neurons into the optic head, then run through the optic

stalk, invading the basal diencephalon at late CS 18.The non-axonal processes of Meitar cells might not only constitute a guidance scaffold for subsequent axonal navigation but also provide a route for molecular communication between brain and optic cup, conceivably playing a part in orchestrating local neurogenesis. In rodents, cats and monkeys, the first axons to enter the optic stalk are from ganglion cells, and before their appearance, the wall of the stalk, like the retina, is composed of undifferentiated neuroepithelium. In ferrets, transient retinopetal axons from the diencephalon enter the optic stalk but do not invade the retina. No cell type comparable to Meitar neurons has been reported in any other mammalian species.

Disclosures: I. Bystron: None. **C. Blakemore:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.07/C25

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant EY012736

Fight for Sight

Prometeo 2012-005

BFU2010-16563

ERC2011-StG201001109

Title: Cyclin d2 controls the generation of a subpopulation of retinal ganglion cells in the ciliary margin zone of the ventral mammalian retina

Authors: *F. MARCUCCI[1], V. MURCIA-BELMONTE[2], E. HERRERA[2], C. MASON[1]; [1]Pathology, Columbia Univ., New York, NY; [2]Inst. de Neurociencias de Alicante, Alicante, Spain

Abstract: The retina of lower vertebrates and chick grows continuously through life by integrating new neurons from a population of proliferating progenitors located at the ciliary margin zone (CMZ). It is unknown whether the mouse CMZ niche provides the neural retina with retinal cells. By performing live imaging in a transgenic mouse line that expresses eGFP in the CMZ and peripheral neural retina, we observed that some cells located in the CMZ move laterally to populate the zone where differentiated retinal ganglion cells (RGC) reside. In

addition, we observed that Cyclin D2, a protein that regulates cell cycle progression, is highly expressed in the proximal CMZ of the ventral retina. Cyclin D2 is required for proliferation and generation of RGCs, in particular, the ipsilaterally projecting RGCs (iRGCs). Interestingly, in the retina of albino mice, which have fewer iRGCs than pigmented retinas, the number of Cyclin D2-positive cells is reduced. Together, these results support the idea that in mammals, the developing CMZ could act as a neurogenic area, giving rise to subsets of RGCs ultimately located in the peripheral neural retina, and that the proper generation of those RGCs depends on the activity of Cyclin D2.

Disclosures: F. Marcucci: None. **V. Murcia-Belmonte:** None. **E. Herrera:** None. **C. Mason:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.08/C26

Topic: A.01. Neurogenesis and Gliogenesis

Support: R01 DC014702

Title: FOXP2 modifies the chromatin landscape of developing neurons

Authors: *S. L. HICKEY, S. BERTO, G. KONOPKA;
Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: FOXP2 is among the few genes implicated in heritable forms of verbal dyspraxia. Because FOXP2 is a transcription factor expressed in the brain during development, its role in regulating developmental pathways that might be important for speech and language has been of particular interest. By analyzing FOXP2 whole genome binding and gene regulation in human neural progenitors (hNPs), we have found evidence that FOXP2 may actively modify the chromatin landscape. We hypothesize that by modifying the chromatin landscape of neural progenitors FOXP2 turns off cellular programs that maintain an undifferentiated state while turning on programs that drive a cell towards a neuronal fate. To further understand the role of FOXP2 in chromatin modification during neural differentiation, we identified areas of nucleosomal depletion using an assay for transposase-accessible chromatin using sequencing (ATAC-seq) in proliferating and differentiating hNPs with and without FOXP2. Using this approach, we found that cells expressing FOXP2 have open chromatin near genes involved in neuron differentiation, and closed chromatin at genes involved in development. Moreover, many of the identified accessible sites are only present in differentiating cells expressing FOXP2,

suggesting that FOXP2 may act as a pioneer factor. Together, these data examine epigenetic regulation by FOXP2 in human neural progenitors over development, a novel role for FOXP2 outside of its previously studied function as a canonical transcription factor.

Disclosures: **S.L. Hickey:** None. **S. Berto:** None. **G. Konopka:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.09/C27

Topic: A.01. Neurogenesis and Gliogenesis

Support: NWO-VICI Grant 865.09.002

Title: Turn-over and function of H3K79 methylation in post-mitotic neuronal development

Authors: *H. V. HEESBEEN;
Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Neuronal progenitors that become post-mitotic still need to follow a long developmental path to finally turn into mature neurons. During this time-window, gene-activity remains heavily regulated, partly by the modification of histones. The roles of histone modificiations in post-mitotic neuronal precursors, when new histones are only introduced by active turnover and not by chromatin duplication, may thus depend on the balance between methylation versus histone turn-over and demethylation. In this study we have focused on Dot1, a methyltransferase that may play a substantial role in the fine-tuning of gene-regulation by facilitating methylation of lysine 79 on histone 3 (H3K79), which levels correlate with transcription. In addition, H3K79 methylation has been proposed to be conservative and no demethylating enzyme has been found thus far. Here, we have investigated the turnover of H3K79 methylation in post-mitotic neurons and sequentially investigated the set of transcripts under regulation of Dot1 activity, the only H3K79 methyltransferase. Herefore we have used mouse models of dopaminergic post-mitotic development and investigated the speed of H3K79 methylation loss in the absence of Dot1. Our data indicates a high turnover of H3K79 methylation. Furthermore, we have found a level-dependent specificity in gene-regulation by Dot1 in post-mitotic dopamine neurons. Finally, loss of Dot function did not affect all dopaminergic neuronal subtypes equally but especially lead to the reduction of substantia nigra dopamine neurons.

Disclosures: **H.V. Heesbeen:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.10/C28

Topic: A.01. Neurogenesis and Gliogenesis

Support: FNR-AFR PhD grant 6669348

Title: Embryonic development of neurons selectively vulnerable in Parkinson's disease

Authors: *M. A. OLIVEIRA, R. BALLING, R. M. T. FLEMING;
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Abstract: A specific set of brainstem nuclei have been proposed to be susceptible to degeneration by histopathological analysis of subjects with suggested prodromal or established early Parkinson's disease. We hypothesise that neuronal vulnerability reflects a shared cellular and molecular phenotypic characteristics that confer selective vulnerability to degeneration. Neuronal cellular and molecular phenotypic specification is mainly the cumulative result of a transcriptional regulatory program active during the development of the nervous system. By manual curation of the developmental biology literature, we comprehensively reconstructed an anatomically resolved cellular developmental lineage of the adult neurons in five brainstem nuclei that are selectively vulnerable to degeneration in early Parkinson's disease. We formed a synthesis of the transcription factors that are required to be active, or required to be inactive, in each of these five brainstem nuclei. Certain transcription factors, e.g., Ascl1 and Lmx1b, seem to required for specification of many neuronal populations that are susceptible to degeneration in early Parkinson's disease. Systematic in vivo assessment of fate determining transcription factors should be completed for all neuronal populations susceptible to degeneration in early Parkinson's disease.

Disclosures: M.A. Oliveira: None. **R. Balling:** None. **R.M.T. Fleming:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.11/C29

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant DE023730

Title: An ancient neurotrophin receptor code, investigating somatosensory neuron diversification in larval zebrafish

Authors: P. GAU, A. CURTRIGHT, D. RAIBLE, *A. K. DHAKA;
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Abstract: Zebrafish have different survival requirements than terrestrial vertebrates. Within days post fertilization (dpf) zebrafish larvae are fully functioning animals that must survive in a hostile environment while they develop into mature fish. As we have reported previously the presence of nociceptive ion channels in all early born TG neurons suggests that nociceptors are the first lineage of somatosensory neurons to develop in fish. This is distinct from terrestrial vertebrates that develop *in ovo* or *in utero* where mechanoceptors and proprioceptors arise during early stages of neurogenesis followed by the formation of nociceptors, suggesting that in zebrafish nociceptors take precedence in larval survival. Notably the expression profile of nociceptive ion channels in zebrafish is consistent with profiles observed in terrestrial vertebrates. These observations argue that fish have substantially different somatosensory requirements than terrestrial vertebrates yet the coding of nociceptor lineages remains largely conserved. During terrestrial vertebrate embryonic development, progenitors differentiate into distinct subclasses that are marked by the unique expression of neurotrophin receptors that are instructive for their survival and specification. Nociceptors, which process pain information, express TrkA; mechanorecepetors which process light touch information, express TrkB and proprioceptors, which measure muscle tension and provide information about limb position, express TrkC. In this study we explore the development of somatosensory neuron populations in larval zebrafish by characterizing the neurotrophin receptor code of sensory neuron populations and investigating genetic programs that shapes somatosensory neuron diversification.

Disclosures: **P. Gau:** None. **A. Curtright:** None. **D. Raible:** None. **A.K. Dhaka:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.12/C30

Topic: A.01. Neurogenesis and Gliogenesis

Support: NWO-Vici Grant 865.09.002

Title: Lmx1b influences the subset specification of mesodiencephalic dopaminergic neurons

Authors: *I. WEVER, P. LARGO BARRIENTOS, M. P. SMIDT;
Mol. Neurosci., Swammerdam Inst. For Life Sci., Amsterdam, Netherlands

Abstract: The Lim Homeobox transcription factor 1 beta (Lmx1b) has been identified as one of the transcription factors that play an important role during the development of mesodiencephalic dopaminergic (mdDA) neurons. During early development, Lmx1b is essential for induction and maintenance of the Isthmic Organizer (IsO), and genetic ablation of this factor results in the disruption of the inductive activity from the IsO and loss of properly differentiated mdDA neurons.
To study the downstream genetic targets of Lmx1b without affecting the IsO, we generated a conditional model in which Lmx1b was selectively deleted in the Pitx3 expression domain from embryonic day (E)11.5 onward. In accordance with literature, no significant changes could be observed in general dopamine (DA) marks, like Th, Pitx3, Vmat2 and En1 at E14.5 and the animals were born at expected Mendelian frequency, were fertile and survived to adulthood. Next to examining the general DA marks, we also investigated several subset marks. The mdDA system can be roughly divided into two groups, namely the substantia nigra Pars Compacta (SNc) and the ventral tegmental area (VTA). Although the two groups originate from the same progenitor pool, they are molecularly different and are differentially depending on transcription factors for their proper development. The rostrolateral group, destined to become the SNc, requires both Pitx3 and En1 to induce the DA phenotype and is closely marked by Ahd2. While the caudal group, destined to become the VTA, is mainly dependent on En1 for the induction of the DA phenotype and is marked by Cck. When looking at these two subgroups at E14.5, we observed a significant up-regulation of the rostrolateral mark Ahd2, while Cck expression is unaffected. When examining other subset marks, like Dat and Calb1, no clear differences were observed, suggesting that Lmx1b might only be important for the regulation of Ahd2. Further studies showed that over-expression of LMX1B in MN9D cells leads to a down-regulation of Ahd2, while expression levels of other factors, like Th and Pitx3, remain unaltered. In addition, we found that Lmx1b can bind to a FLAT element in the Ahd2 gene, suggesting that Lmx1b might influence Ahd2 expression directly by binding to this element.

Disclosures: I. Wever: None. **P. Largo Barrientos:** None. **M.P. Smidt:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

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Topic: A.01. Neurogenesis and Gliogenesis

Support: CONICET

ANPCyT PICT-2014-1821 and PICT-2014-3167

ISN

Title: Transcriptional control of the specification of spinal CSF-contacting Neurons

Authors: D. J. DI BELLA[1], A. L. CARCAGNO[1], M. B. PARDI[2], M. SARTORETTI[1], L. BRUM[1], A. MARÍN-BURGIN[2], *G. M. LANUZA[1];
[1]Inst. Leloir, Buenos Aires, Argentina; [2]IBIOBA, Buenos Aires, Argentina

Abstract: The generation of precise neuronal types in the right time and quantity, is essential for building a functioning nervous system. In the last 20 years, we have reached a huge understanding of the genetic mechanisms that control neuron specification during embryonic development, in which extrinsic cues are translated into positional coordinates that determine neuronal identity. However, the temporal contribution to neuronal diversity has been less explored. We have recently identified a neurogenic event in the amniote spinal cord that takes place simultaneously with glial specification, during advanced developmental stages previously considered non-neurogenic. CerebroSpinal Fluid contacting Neurons (CSF-cN), widely conserved in chordates, are located at the interface between the central nervous system and the CSF and are generated from late ventral progenitors. The genetic mechanisms that allow the differentiation of this class of neurons at gliogenic stages are unknown. In this work, we identified that the transcription factors Ascl1, Gata3 and Gata2 are sequentially expressed in mouse CSF-cN and control their specification. Through expression analysis and mouse genetics, we described that Gata3 and Gata2 are postmitotically expressed in CSF-cN, where they control the acquisition of this neuronal identity. Loss of function experiments showed that Gata3 and Gata2 play distinct roles in different CSF-cN subsets. Meanwhile, the proneural protein Ascl1 is expressed in late ventral proliferating progenitors that give rise to CSF-cN and is exclusively necessary for their differentiation in amniotes. By temporal dissection of Ascl1 activity, we found that this transcription factor is an essential component of late CSF-cN neurogenesis, acting at the time of their specification. Finally, fate mapping experiments in the absence of Ascl1 demonstrated that it confers neurogenic potential to late ventral progenitors, which would otherwise become ependymal cells. We conclude that the sequencial action of Ascl1-Gata3/2 directs the late specification of CSF-cN in amniotes. Ascl1 acts governing the onset of neuronal differentiation in the gliogenic neural tube and Gata3/2 control CSF-cN identity.

Disclosures: **D.J. Di Bella:** None. **A.L. Carcagno:** None. **M.B. Pardi:** None. **M. Sartoretti:** None. **L. Brum:** None. **A. Marín-Burgin:** None. **G.M. Lanuza:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NRF-2015R1A2A1A15055611

NRF-2012M3A9C6050508

GCRC 2011-0030001

Title: Identification of STAM1 as a novel effector of ventral projection of spinal motor neurons

Authors: ***S. LEE**, H. NAM;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: During spinal cord development, motor neuron (MN) axons exit the spinal cord ventrally, although the molecular basis for this process remains poorly understood. STAM1 and Hrs form a complex involved with endosomal targeting of cargo proteins, including the chemokine receptor CXCR4. Interestingly, the absence of CXCR4 signaling in spinal MNs is known to enforce improper extension of the axons into the dorsal side of the spinal cord. Here we report that the MN-specific Isl1-Lhx3 complex directly transactivates the *Stam1* gene and STAM1 functions in determining the ventral spinal MN axonal projections. STAM1 is co-expressed with Hrs in embryonic spinal MNs, and knock-down of STAM1 in the developing chick spinal cord results in down-regulation of the expression of CXCR4, accompanied by dorsally projecting motor axons. Interestingly, overexpression of STAM1 or CXCR4 also results in dorsal projection of motor axons, suggesting that proper CXCR4 protein level is critical for the ventral motor axon trajectory. Our results reveal a critical regulatory axis for the ventral axonal trajectory of developing spinal MNs, consisting of the Isl1-Lhx3 complex, STAM1 and CXCR4.

Disclosures: **S. Lee:** None. **H. Nam:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.15/C33

Topic: A.03. Stem Cells and Reprogramming

Support: CIRM (RB5-07320)

National Institutes of Health (NS 063999)

Title: The mechanistic basis by which the Bone Morphogenetic Proteins direct sensory interneuron identity in the dorsal spinal cord

Authors: *M. ANDREWS, L. DEL CASTILLO, D. SIVALINGAM, S. J. BUTLER; Neurobio., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Inductive signaling by the Bone Morphogenetic Protein (BMP) family is reiteratively required to direct stem and progenitor cells towards different cellular fates throughout embryonic development. This family of growth factors is thus a critical reagent used in many stem cell replacement therapies. As part of the effort to develop stem cell-based approaches to rebuild damaged or diseased spinal cords, we are studying how the BMPs direct neuronal identity in the embryonic spinal cord. The roof plate (RP), at the dorsal midline of the spinal cord, expresses many BMPs and the collective activity of these BMPs is required to specify different populations of sensory interneurons (INs) in the adjacent dorsal spinal cord. Surprisingly, the mode by which the BMPs direct IN identity remains unresolved, and there is no established protocol to derive these classes of spinal sensory INs in vitro. Previous studies have suggested that the BMPs act as concentration-dependent morphogens to direct neural identity, largely by analogy with the Sonic hedgehog gradient that patterns the ventral spinal cord. However, it is unclear how multiple BMPs would cooperate to establish a single morphogen gradient. Moreover, our recent studies have suggested that different BMPs have distinct effects on the induction of particular IN fates. Using both in vitro and in vivo methods, we are assessing the extent to which the different BMPs act in a concentration dependent manner, or have signal-specific activities in directing cellular identity. Additionally, we are evaluating how the canonical BMP second messengers, the Smad proteins, translate the activities of the different BMPs into the specification of particular neural identities. Through these studies, we will better understand the mechanism by which the BMPs direct IN identity during development while also developing a stem cell differentiation protocol as a first step towards the restoration of sensory circuitry after spinal damage.

Disclosures: **M. Andrews:** None. **L. Del Castillo:** None. **D. Sivalingam:** None. **S.J. Butler:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.16/C34

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant 4R00NS084988 - 03

Title: Hox proteins generate neuronal diversity by regulating the transcriptional output of a single terminal selector gene

Authors: *P. KRATSIOS[1], S. YEN KERK[2], O. HOBERT[2];
[1]Neurobio., Univ. of Chicago, Chicago, IL; [2]Columbia Univ., New York, NY

Abstract: Understanding how the expression of neuron class-specific genes is established during development is of key importance to the problem of neuronal diversity. Exploiting the molecular diversity of distinct motor neuron (MN) classes in *C.elegans*, we show that the conserved COE-type terminal selector UNC-3 is required for MN diversity by directly regulating the expression of MN class-specific terminal identity genes. We further find that Hox proteins cooperate with UNC-3 and remarkably employ two distinct, region-based strategies to generate MN diversity. In the *C.elegans* ventral nerve cord, the more anteriorly expressed *Hox* genes, *lin-39*/Scr/Dfd and *mab-5* (Antennapedia-type), act as UNC-3 coactivators and directly regulate the expression of MN class-specific genes. Conversely, the posterior *Hox* gene *egl-5/Abd-B* represses *lin-39* and *mab-5 expression*, thereby diversifying posteriorly located MNs. Intriguingly, *Hox* and *unc-3* orthologs are coexpressed in mouse MNs along the spinal cord, suggesting that this intersectional, region-based regulatory principle may be conserved across phylogeny.

Disclosures: **P. Kratsios:** None. **S. Yen Kerk:** None. **O. Hobert:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.17/D1

Topic: B.12. Glial Mechanisms

Support: NSF GRFP

Title: Effects of response learning on medium spiny neurons and astrocytes in the dorsal striatum

Authors: *B. A. BRIONES[1,2], E. GOULD[1,2];
[1]Psychology, [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: In the rodent, the dorsolateral striatum (DLS) is required for motor-response learning (Packard & Knowlton, 2002). Despite strong evidence linking the DLS with response learning, little is known about whether the region is structurally plastic in response to engagement in response learning tasks. Such information would fill in the gaps in our knowledge of how the striatum responds to experience and may provide clues about the mechanisms underlying response learning. To investigate this question, we used DiI labeling to analyze dendritic spines, primary sites of excitatory synapses, on medium spiny neurons. Our preliminary results suggest that response learning increases dendritic spine density and head width in the DLS and produces greater immediate early gene expression in the DLS compared to the dorsomedial striatum, a region implicated in spatial-place learning. Next, since it is becoming increasingly clear that neurons work in concert with nonneuronal cells to mediate synaptic function and plasticity (Barres, 2008), we examined whether morphological differences exist in GFAP-labeled astrocytes after dorsal striatum dependent behavior and found that GFAP-labeled astrocyte number and size was greater in response learning animals compared to controls. Future studies will seek to understand the functional consequences and region-specificity of these differences as well as the potential role of astrocytes and astrocyte-neuron interactions in response learning.

Disclosures: B.A. Briones: None. **E. Gould:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.18/D2

Topic: A.01. Neurogenesis and Gliogenesis

Support: HFSP-RGP0063

Title: Genetic analysis of KA and V2b neuron specification in zebrafish spinal cord

Authors: *S. BANERJEE[1], L. ANDRZEJCZUK[1], S. J. ENGLAND[1], A. PRENDERGAST[2], L. DESBAN[2], S. HARVEY[3], H. WILLIAMS[3], R. KETTLEBOROUGH[3], C. WYART[2], K. E. LEWIS[1];

[1]Syracuse Univ., Syracuse, NY; [2]INSTITUT DU CERVEAU ET DE LA MOELLE EPINIERE, Paris, France; [3]Wellcome Trust Sanger Inst., Cambridge, United Kingdom

Abstract: Coordinated motor behavior is critically dependent on neural circuits that form within the ventral spinal cord. Three types of GABAergic interneurons that are important components of these circuits are V2b interneurons and two classes of Kolmer Aghdur (KA) CSF contacting neurons (KA' and KA'' cells). These three types of neurons originate from three distinct progenitor domains in the ventral spinal cord. Although recent research has identified some physiological functions and behavioral roles of these interneurons, little is known about how these cells are functionally specified during development. V2b and KA neurons express post-mitotic transcription factors Gata2, Gata3 and Tal1. Using mutants for each of these genes we have shown that these three transcription factors have unique roles in determining the GABAergic identity and regulating the expression of several other transcription factor genes in V2b and KA neurons. Interestingly, we found that even though Gata2, Gata3 and Tal1 are expressed in all three cell types, the phenotypes of each of the three mutants affect different subsets of KA and V2b cells.
To identify additional genes that may be involved in specifying functional properties of KA neurons, we have isolated pure populations of these cells using FACS and performed RNAseq analyses. We have identified 293 genes that are enriched in KA neurons compared to a pan neuronal population. We are analyzing these genes by expression (*in situ* hybridization) and functional (loss of function mutant) studies. Together, data from these studies will provide a more comprehensive understanding of the development of V2b and KA/CSF contacting neurons.

Disclosures: S. Banerjee: None. **L. Andrzejczuk:** None. **S.J. England:** None. **A. Prendergast:** None. **L. Desban:** None. **S. Harvey:** None. **H. Williams:** None. **R. Kettleborough:** None. **C. Wyart:** None. **K.E. Lewis:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

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Topic: A.01. Neurogenesis and Gliogenesis

Support: GVSU Student Summer Scholars Program

West Michigan Science and Technology Initiative

Michigan Space Grant Consortium

Title: Investigation of dopaminergic neuron lineage marker expression from human nato3 overexpression in the developing chick embryo

Authors: *D. DOYLE, J. KOELSCH, N. HUISINGH, M. TAYLOR;
Grand Valley State Univ., Allendale, MI

Abstract: In Parkinson's disease, the loss of mesencephalic dopaminergic neurons within the pars compacta region of the substantia nigra could be ameliorated with cell replacement therapy. There is no clear answer as to how Nato3, a basic helix-loop-helix transcription factor expressed in the floorplate of the developing midbrain, affects the generation of dopaminergic neurons in the developing nervous system. Previous studies in our lab have investigated the effects of an overexpression of Nato3 from *Mus musculus* in the developing chick embryo. We found that overexpressing Nato3 sufficiently induces ectopic expression of the floor plate cell markers Shh and Foxa2 in the developing midbrain, as well as the immature dopamine neuron marker Lmx1b in the developing midbrain and spinal cord. Through the use of *in ovo* electroporation of a bicistronic EGFP reporter expression vector, and immunohistochemistry we are currently examining the effects of overexpression of Nato3 from *Homo sapiens* in the developing chick embryo. The results are expected to be similar to that obtained with Nato3 from *Mus musculus* based on 100% identity between the two protein sequences within the loop region of the transcription factor. The previous information obtained with Nato3 from *Mus musculus* provides evidence that Nato3 affects cells within a dopaminergic neuron lineage. Characterization of the effects of the overexpression of Nato3 from *Homo sapiens* could identify possible uses related to the development of dopaminergic neurons for cell therapy.

Disclosures: D. Doyle: None. **J. Koelsch:** None. **N. Huisingh:** None. **M. Taylor:** None.

Poster

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Program#/Poster#: 493.20/D4

Topic: A.01. Neurogenesis and Gliogenesis

Title: Prostaglandin E2 promotes neurite outgrowth via EP2-cAMP signaling pathway in NSC-34 cells, a motor neuron-like cell line.

Authors: *H. NANGO, Y. KOSUGE, K. ISHIGE, Y. ITO;
Lab. of Pharmacol., Sch. of Pharmacy, Nihon Univ., Funabashi-Shi, Japan

Abstract: Prostaglandin E2 (PGE2), one of the major lipid mediators, exerts various biological effects by binding to 4 subtypes of E-prostanoid receptors (EP1-4). In addition, it is well known that PGE2 induces cell proliferation and differentiation in a number of different cells. However, the effects of PGE2 on cell proliferation and differentiation of motor neurons have not been clarified. The purpose of this study was to elucidate the mechanism of PGE2-induced differentiation of motor neurons using NSC-34 cells, a mouse motor neuron-like cell line.
We first examined whether exogenously applied PGE2 can suppress cell proliferation in undifferentiated NSC-34 cells using MTT reduction assay. Exposure of these cells to PGE2 (1-100 μM) for 48 h resulted in a reduction of MTT activity in a concentration-dependent manner. Immunoblotting studies showed that EP2 and EP3 were dominantly expressed in NSC-34 cells as well as motor neurons in mice. In order to clarify the subtype of EP receptors that contribute to effects of PGE2 in the cells, we investigated effects of two different types of EP agonists on cell proliferation in these cells. Treatment of these cells with butaprost (1-20 μM), an EP2-selective agonist, resulted in a concentration-dependent decrease of MTT reduction, and this effect was stronger than that that of PGE2. In contrast, sulprostone (1-30 μM), an EP1/3 agonist, had no effect. Next, we observed morphological transformation with phase-contrast microscope to evaluate the effects of PGE2 and these EP agonists on neurite outgrowth, and found that PGE2 and butaprost but not sulprostone promoted neurite outgrowth and increased the number of neurite-bearing cells. We also investigated the cytotoxic effects of PGE2 and butaprost on undifferentiated NSC-34 cells by propidium iodide (PI) fluorescent staining. Exposure of these cells to PGE2 and butaprost for 48 h did not increase the number of PI-positive cells. Dibutyryl-cAMP (1 mM), a cAMP analog, increased the number of neurite-bearing cells with no effect on cell proliferation in these cells.
In conclusion, the present study showed that PGE2 promotes neurite outgrowth and suppresses cell proliferation via EP2 subtype, and that cAMP-signaling pathway is involved in PGE2-induced differentiation of NSC-34 cells.

Disclosures: **H. Nango:** None. **Y. Kosuge:** None. **K. Ishige:** None. **Y. Ito:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.21/D5

Topic: A.01. Neurogenesis and Gliogenesis

Support: HRC

Title: Temporal transcription factor expression delineates lateral hypothalamic neurogenesis.

Authors: *H. R. TWIGG, C. L. JASONI;
Univ. of Otago, Dunedin, New Zealand

Abstract: There is clear evidence for a link between maternal obesity during pregnancy and the offspring's heightened risk for disease in later life, including obesity and the metabolic syndrome. This effect has been replicated in numerous rodent and primate models of maternal obesity. The hypothalamus is the key player in the neural regulation of body weight and energy homeostasis. Neurons of the hypothalamus receive endocrine signals from the body and begin to coordinate appropriate behavioural and physiological responses. Melanin-concentrating hormone (MCH)-producing neurons of the lateral hypothalamus (LH) are orexigenic, and contribute to body weight regulation by promoting feeding and consummative behaviours, amongst other homeostatic roles. We and others found an increase in expression of *pmch*, the mRNA encoding MCH, in offspring from obese rodent dams. However, whether this was due to an increase in the number of *pmch*-expressing cells, or an increase in the amount of *pmch* RNA expressed per cell was unclear. We have recently observed an increase in the number of MCH-expressing cells in offspring from obese dams. We hypothesise that this change in cell number occurs due to altered expression of developmental genes in the developing hypothalamus, such that hypothalamic progenitor cells produce increased numbers of differentiated MCH-expressing cells. To address this hypothesis, we first searched online resources to select transcription factors with putative binding sites at conserved regions of *pmch*, and which are known to be expressed in the developing hypothalamus. These transcription factors include *Dbx1,* which has a known role in MCH development, *Sim1,* and *FoxO1.* RNA was collected from the developing LHA from both wild-type mouse embryos, and embryos from obese mothers at gestational days (GD) 11.5, 14.5, 16.5 and 18.5, and transcription factor expression was assessed using quantitative PCR (qPCR). *FoxO1* showed stable expression across normal development. *Sim1* showed low levels of expression at GD11.5 but gradually increased to peak at GD18.5. *Dbx1* showed low levels of expression at GD11.5, peaked dramatically with a 16-fold increase at GD14.5, dropping again to GD11.5 levels by GD18.5. Maternal obesity during pregnancy affected the normal expression profile of these genes in the fetal hypothalamus. Perturbation to the expression profiles of transcription factors required for normal LH and MCH cell development, influenced by maternal obesity, may therefore contribute to an altered fate of hypothalamic progenitors, and thus to an increase in orexigenic MCH neurons.

Disclosures: H.R. Twigg: None. **C.L. Jasoni:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.22/D6

Topic: A.01. Neurogenesis and Gliogenesis

Support: National Natural Science Foundation of China for Young Scholars (31400923)

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Shenzhen Knowledge Innovation Program (JCYJ20151030140325152)

Shenzhen Peacock Plan

Title: Coronin 2B is an important regulator of neuronal polarization

Authors: *Y. CHEN[1,2,3,4,5], Y. CHEN[1,2,3,4,5], Y. ZHANG[1], A. FU[1,2,3,4], N. IP[1,2,3,4];
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Abstract: The establishment of neuronal polarity is vital for neuronal migration, the wiring of neuronal circuits, and synaptic connectivity; the disruption of these processes leads to various neurodevelopmental disorders such as autism spectrum disorder, mental retardation, and schizophrenia. Well-orchestrated extracellular and intracellular signaling cascades shape neuronal polarization, and actin cytoskeleton reorganization is of particular importance. Here, we investigated the function of Coronin 2B, a member of an evolutionarily conserved actin-binding protein family, during neuronal polarization. Coronin 2B was enriched in the mouse nervous system, specifically in the cerebral cortex and hippocampus. In early differentiating cultured hippocampal neurons, Coronin 2B was concentrated at neurite tips and co-localized with F-actin. Silencing Coronin 2B expression in hippocampal neurons by shRNA significantly reduced the number of MAP2-positive dendrites, while overexpression increased the number of MAP2-positive dendrites, accompanied by the loss of tau-positive axons. These findings strongly suggest that Coronin 2B is a key regulator of neuronal polarization. Further understanding of the roles of Coronin 2B in the establishment of neuronal polarity may provide clues on its functions in central nervous system development.

Disclosures: **Y. Chen:** None. **Y. Chen:** None. **Y. Zhang:** None. **A. Fu:** None. **N. Ip:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

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Program#/Poster#: 493.23/D7

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant HD086820

NIH Grant MH110160

Title: Clonal production and organization of thalamic neurons.

Authors: *Q.-F. WU[1], S. WONG[2], E. P. SCOTT[3], M. FREEMAN[3], C. JOU[2], B. YOUNG[2], V. PUROHIT[2], G.-L. MING[2], Y. NAKAGAWA[3], H. SONG[2];
[1]Inst. of Cell Engineering, Dept. of Neurol., [2]Inst. for Cell Engin., Johns Hopkins Med. Sch., Baltimore, MD; [3]Dept. of Neurosci., Univ. of Minnesota Med. Sch., Minneapolis, MN

Abstract: The cellular production, distribution and migration dictates the architectonic organization of brain, which includes both stratified (e.g. neocortex) and nuclear (e.g. thalamus, hypothalamus and brain stem) structures. In contrast to the well-studied stratified neocortex, little is known about the cellular production and organization in the nuclear structures. Using multiple driver lines including Gli1-CreER^{T2}, Axin2-CreER^{T2}, Neurog1-CreER^{T2} or Olig3-CreER^{T2} mice in combination with Mosaic Analysis with Double Markers (MADM)-based multicolor reporter system, we trace the ontogenetic clonal units arising from individual neuroepithelial cells (NEs) or intermediate progenitor cells (IPCs) to determine the cellular production and organization in the thalamus. Clonal analysis uncovers that Gli1- and Axin2-labeled NEs generate more progeny cells than Olig3-labeled population, suggesting Shh- and Wnt-responding populations of NEs with higher potency in proliferation. The clonally-related neurons generated by individual NEs are typically organized into radial columns, which specify a specific set of thalamic nuclei. There are no significant differences in cellular distribution and organization among Gli1-, Axin2- and Olig3-labeled clones. A majority of Neurog1-labeled clones contain 4 or fewer cells but they still populate different nuclei, indicating a late specification of nuclear fate. Taken together, our study reveals the NEs building the thalamus are heterogeneous in generating progeny cells but utilize uniform principles to organize sibling thalamic neurons.

Disclosures: Q. Wu: None. **S. Wong:** None. **E.P. Scott:** None. **M. Freeman:** None. **C. Jou:** None. **B. Young:** None. **V. Purohit:** None. **G. Ming:** None. **Y. Nakagawa:** None. **H. Song:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

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Program#/Poster#: 493.24/D8

Topic: B.12. Glial Mechanisms

Support: National Council of Science and Technology of Mexico (CONACYT) grant FC-251

Title: Analysis of the effects of vasoinhibins on hippocampal neurons

Authors: *R. M. AROÑA[1], E. ARNOLD[1,2], F. MACÍAS[1], C. CLAPP[1], G. MARTÍNEZ DE LA ESCALERA[1];
[1]Inst. de Neurobiología, Univ. Nacional Autónoma de México (UNAM), Querétaro, Mexico; [2]Catedrática CONACYT-Instituto de Neurobiología, Univ. Nacional Autónoma de México (UNAM), Querétaro, Mexico

Abstract: Vasoinhibins (Vi) are a family of peptides derived from the hormone prolactin that have been shown to act on endothelial cells inhibiting angiogenesis, vasodilation and vasopermeability. Furthermore, Vi can participate in the modulation of some functions of the central nervous system (CNS) such as stimulating vasopressin secretion and promoting anxiety and depression behaviors. The hippocampus has been implicated in these behaviors; thus, in the present study we explored whether Vi are generated in this structure and if they affect hippocampal neurons. Extracts from hippocampus were evaluated for the presence of Vi as well as for the intrinsic capacity of this tissue to cleave prolactin to generate Vi. To explore the actions of Vi on hippocampal neurons, primary fetal hippocampal neuron-enriched cultures were isolated from the brain of E16 mice and seeded on plates treated with poly-L-lysine. Hippocampal cultures were treated on the first day in vitro (DIV1) with increasing concentrations of Vi (5-20 nM) for up to 72 hours (DIV2-DIV4). Vi were found in the hippocampus, and incubation of prolactin with extracts from hippocampus resulted in the proteolysis of this hormone to yield Vi, both revealed by Western blot. Incubation of hippocampal neuron-enriched cultures with Vi reduced in a dose-dependent manner the cell number, as well as the metabolic activity, evaluated by microphotography image analysis and MTT assay, respectively. Altogether these findings show that Vi are produced locally in the hippocampus and are able to affect its neurons, suggesting a possible site for the reported actions of Vi on anxiety and depression.

Disclosures: R.M. Aroña: None. **E. Arnold:** None. **F. Macías:** None. **C. Clapp:** None. **G. Martínez de la Escalera:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

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Program#/Poster#: 493.25/D9

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH AA021402

The Shaffer Family Foundation

NIH 5T32AG000216-23

Title: Genomically mosaic neurons of the adult mouse brain identified by DNA content variation (DCV)

Authors: *B. A. SIDDOWAY[1,2], S. ROHRBACK[2], C. SAUVEY[2], G. KAESER[2], A. CHEN[2], A. CERDA[2], M.-H. LEE[2], J. CHUN[2];
[1]Neurosci., LSU Hlth. Sci., New Orleans, LA; [2]The Scripps Res. Inst., San Diego, CA

Abstract: Recent reports have determined that neurons from the same individual display substantial genomic variability among themselves and from germline. These newer studies confirm older work and hypotheses regarding the existence of genomically mosaic neurons in the brain. The physiological role of genomic mosaicism in neuronal populations remains unknown, however, genomic variability appears to be a standard feature of mature neurons in the normal brain and is consistently increased in sporadic Alzheimer's disease (SAD). Mosaic genomic differences among human neurons occur in many forms, including aneuploidies, copy number variations, repeat domain variability, LINE element insertions, single nucleotide variations, and global variability in DNA content (DNA content variation (**DCV**)) occurring both during and after neurodevelopment. It has been less clear whether DCV is present in the mouse brain. Here we report results from whole genome DNA content analysis in several mouse lines that indicate **DCV** exists and is enriched in neuronal subpopulations when compared across both brain regions and cell types. These results were validated by examining total DNA yield of flow sorted nuclei from each subgroup as well as via a semi-quantitative whole genome amplification approach. We have additionally developed and optimized single-cell sequencing methodology to examine mosaic genomic variation among individual neurons from adult mouse hippocampus. The presence of DCV and genomic variability in mouse neurons complements data from human brain and represents an animal model to investigate and characterize genomic mosaicism in the central nervous system.

Disclosures: **B.A. Siddoway:** None. **S. Rohrback:** None. **C. Sauvey:** None. **G. Kaeser:** None. **A. Chen:** None. **A. Cerda:** None. **M. Lee:** None. **J. Chun:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Duncan and Nancy MacMillan Developing Chair

Decibel Therapeutics

Anne B. & James B. Leathem Fellowship

TA/GA Development Fund

Title: Neurog1 transcriptional activity is dependent on cellular context

Authors: ***C. SONG**, K. KWAN;
Cell Biol. and Neurosci., Rutgers Univ., Piscataway, NJ

Abstract: Loss of spiral ganglion neurons (SGNs) is a major cause of hearing loss. There are currently no available treatments for SGN loss. Understanding the role of key transcription factors that promote SGN differentiation will accelerate efforts for stem cell replacement therapies. Neurog1, a pro-neural transcription factor, is highly expressed in the neural-sensory-competent domain (NSD) of the developing inner ear. Neurog1 is required for generation of SGNs during otic neurogenesis. Repurposing Neurog1 activity is a potential way to promote SGN regeneration. We employ a immortalized multipotent otic progenitor (iMOP) cell line that can self-renew or differentiate into SGNs depending on various molecular cues. By overexpression or knockdown of Neurog1 in iMOP cells, we determined that Neurog1 promotes proliferation in the multipotent progenitors and confers neuronal fate during differentiation by promoting expression of different downstream targets. ChIP-seq experiments of active(H3K4me3) and repressive(H3K27me3) chromatin marks suggest that Neurog1 target genes may be transcriptionally regulated. In conclusion, our findings show that the function of Neurog1 is context dependent and can partly be attributed to the epigenetic status of its downstream targets.

Disclosures: **C. Song:** None. **K. Kwan:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.27/D11

Topic: A.01. Neurogenesis and Gliogenesis

Support: Pritzker Neuropsychiatric Research Consortium

ONR: N00014-12-1-0366

NIH: R01MH104261

Title: Both FGF2- and protein kinase C-mediated signaling determine differentiation of SH-SY5Y human neuroblastoma cells.

Authors: *L. A. DOKAS, S. J. WATSON, H. AKIL;
Univ. of Michigan, Ann Arbor, MI

Abstract: The SH-SY5Y cell line is an N-type neuroblastoma with catecholaminergic properties. These cells can be further differentiated by several mechanisms including the combination of a growth factor and a phorbol ester that activates protein kinase C (PKC). One of the most effective growth factors in this regard is FGF2. Given the phenotype of SH-SY5Y cells, differentiation with FGF2 and phorbol ester may model development from a neuroblastic stage to more mature neuronal morphology. We have previously reported that FGF2 and the phorbol ester, phorbol 12, 13-dibutyrate (PDB) each affect the morphology of differentiating cells: PDB produces lamellipodial profiles while FGF2 elongates cells and processes. In combination, highly networked cells result. In order to understand the signaling that underlies these changes, interactions between FGF2 and PKC are being characterized in undifferentiated and differentiated cells. Both FGF2 and PDB activate the extracellular stimulus-regulated kinase1/2 (ERK1/2) pathway, measured as phosphorylation of the activation domain of ERK1/2 at Thr202/Tyr204, with a considerable degree of crosstalk in undifferentiated cells. Although only the effect of FGF2 is blocked by the FGF receptor (FGFR)-selective inhibitor, PD 173074, the PKC inhibitor, GF 109203X, blocks both PDB- and FGF2-mediated ERK1/2 phosphorylation. One likely site of this crosstalk is at the level of raf since inhibition of MEK1/2, the protein kinase target of raf, affects ERK1/2 phosphorylation in response to either stimulus. Furthermore, ERK1/2 phosphorylation in response to FGF2 is lost in differentiated cells indicating that a change in FGFR sensitivity or coupling occurs during the process of differentiation. As a second means of characterizing involvement of PKC in SH-SY5Y cell differentiation, phosphorylation of two major substrate proteins, myristoylated alanine-rich C kinase substrate (MARCKS) and growth-associated protein-43 (GAP-43) have been compared. Both expression and phosphorylation of GAP-43 at Ser41 are increased in differentiated cells, principally in response

to PDB. MARCKS phosphorylation at Ser167/170 is also PDB-sensitive; however, in this case, co-exposure to FGF2 reduces levels of phospho-MARCKS. Since both MARCKS and GAP-43 are involved in actin-based remodeling of the neuronal cytoskeleton, differential regulation of their phosphorylated states by PKC and FGF2 could underlie the changes in cell morphology seen during differentiation of SH-SY5Y cells.

Disclosures: L.A. Dokas: None. **S.J. Watson:** None. **H. Akil:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

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Program#/Poster#: 493.28/D12

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH P41-GM103540

NIH P50-GM076516

Title: Assessment of membrane fluidity changes during cellular development reveals time and cell type specificity

Authors: *P. NOUTSI[1], E. GRATTON[2], S. CHAIEB[3];
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Abstract: Cell membrane is made up of a complex structure of lipids and proteins that diffuse laterally giving rise to what we call membrane fluidity. During cellular development such as neuronal differentiation cell membranes undergo dramatic structural changes induced by proteins such as ARC and Cofilin among others in the case of synaptic modification. In this study we used the generalized polarization (GP) property of fluorescent probe Laurdan using two-photon microscopy to determine membrane fluidity as a function of time and for various cell lines. A low GP value corresponds to a higher fluidity and a higher GP value is associated with a more rigid membrane. Four different cell lines were monitored such as hN2, NIH3T3, HEK293 and L6 cells. Membrane fluidity was measured at 12h, 72h and 92 h. Our results show significant changes in membrane fluidity among all cell types at different time points. GP values tend to increase significantly within 92 h in hN2 cells and 72 h in NIH3T3 cells and at 92 h in HEK293 cells. L6 showed a marked decrease in membrane fluidity at 72 h and starts to increase at 92 h. As expected, NIH3T3 cells have more rigid membrane at earlier time points. On the other hand neurons tend to have the highest membrane fluidity at early time points emphasizing its

correlation with plasticity and the need for malleability during development. This study sheds light on the involvement of membrane fluidity during development in neurons and other cell lines

Disclosures: **P. Noutsi:** None. **E. Gratton:** None. **S. Chaieb:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

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Program#/Poster#: 493.29/D13

Topic: A.01. Neurogenesis and Gliogenesis

Support: R01AA024659

TAMU Graduate Merit Fellowship

Title: Ethanol exposure induces Id gene expression in neural stem cells

Authors: ***A. TSENG**[1], N. SALEM[1], S. BURROWES[1], S. BALARAMAM[1], A. MAHNKE[1], S. EAVES[2], M. RAVEN[2], C. GARCIA[2], C. BEDDINGFIELD[2], R. MIRANDA[1];
[1]Mol. & Cell. Med., Texas A&M Hlth. Sci. Ctr., College Station, TX; [2]Texas A&M Univ., College Station, TX

Abstract: Prenatal alcohol exposure can result in a cluster of craniofacial, neuro-cognitive and growth deficits that are collectively termed Fetal Alcohol Spectrum Disorders (FASD). FASD is difficult to prevent and is the leading non-genetic cause of neurodevelopment disability worldwide. Neural stem cells (NSCs) are particularly vulnerable to alcohol (ethanol) exposure during the late first through the second trimester, when they are most extensively involved in neurogenesis. We previously found that ethanol induced premature depletion and aberrant maturation of NSCs. The mechanisms underlying aberrant NSC maturation are poorly understood. We hypothesized that the ID (Inhibitor of DNA binding) protein family was a potential mediator of ethanol induced NSC dysfunction. All four members of this highly conserved protein family regulate differentiation of NSCs during development and in the adult, by binding and inhibiting the activity of a number of basic helix-loop-helix transcription factors. We report mRNA transcripts of three out of the four ID protein family members are significantly decreased during neural differentiation, and furthermore, that ethanol increased expression of these transcripts in fetal NSCs. We further confirmed elevated protein expression of ID1 following ethanol exposure. Heuristic prediction of ID1 binding partners coupled with Gene Ontology (GO) analysis shows that ID1 targets are significantly enriched for determinants of

neural differentiation. Therefore, ethanol induced ID protein expression may underlie some of the aberrant neural phenotypes resulting from fetal alcohol exposure.

Disclosures: **A. Tseng:** None. **N. Salem:** None. **S. Burrowes:** None. **S. Balaramam:** None. **A. Mahnke:** None. **S. Eaves:** None. **M. Raven:** None. **C. Garcia:** None. **C. Beddingfield:** None. **R. Miranda:** None.

Poster

493. Mechanisms of Neuronal Differentiation and Fate Specification

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 493.30/D14

Topic: A.01. Neurogenesis and Gliogenesis

Title: Effects of different BMP receptor subunits on gene expression in neural progenitor cells

Authors: ***J. CHEN**[1], C.-Y. PENG[2], J. A. KESSLER[2];
[2]Neurol., [1]Northwestern Univ., Chicago, IL

Abstract: Bone morphogenetic protein (BMP) signaling regulates neural stem and progenitor cell (NPC) maturation. BMP receptors are heterotetramers composed of two BMP receptor II (BMPRII) subunits and two of either BMP receptor 1a (BMPR1a) or BMP receptor 1b (BMPR1b) subunits. Although BMPR1a and BMPR1b have similar signal transduction pathways, they exert different effects on NPC proliferation and differentiation. To examine the molecular mechanisms underlying these differences, we sequenced the RNA transcriptomes of wild-type, $BMPRII^{fx/fx}$, $BMPR1a^{fx/fx}$, and BMPR1b-/- cultured postnatal subventricular zone neurospheres treated either with BMP4 or with the BMP inhibitor, noggin. Although BMPR1a and BMPR1b have overlapping downstream signaling pathways and targets, RNA deep sequencing showed many differences in the genes they regulate. After 4 hours of BMP4 treatment, 5391 genes were significantly upregulated in wild-type neurospheres, 2183 genes in $BMPRII^{fx/fx}$ neurospheres, 1471 genes in $BMPR1a^{fx/fx}$ neurospheres, and 2737 in BMPR1b-/- neurospheres. When $BMPR1a^{fx/fx}$ and BMPR1b-/- gene lists are compared, $BMPR1a^{fx/fx}$ neurospheres have 260 genes uniquely regulated in response to BMP4 treatment, while BMPR1b-/- neurospheres have 1526 genes uniquely regulated. Using pathway analysis tools, we found that MAPK signaling, Wnt signaling, and heterotrimeric G-protein signaling are influenced by BMP4 treatment. Gene ontology analysis shows several processes differentially regulated by BMP4 treatment of $BMPR1a^{fx/fx}$ and BMPR1b-/- neurospheres, including Wnt signaling, synaptic signaling, and gliogenesis. These data support previous evidence that BMPR1a and BMPR1b activate different downstream signals in NPCs, as well as identify new BMP transcriptional targets for future study.

Disclosures: **J. Chen:** None. **C. Peng:** None. **J.A. Kessler:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.01/D15

Topic: A.02. Postnatal Neurogenesis

Support: 31-116689

310030_135736/1

51AU40_125759

Title: Effect of redox dysregulation on adult hippocampal neurogenesis in a mouse model of schizophrenia

Authors: *B. GIANGRECO[1], D. DWIR[1], J. KOCHER-BRAISSANT[2], J.-H. CABUNGCAL[1], S. SULTAN[2], P. STEULLET[1], E. GEBARA[2], R. CHRAST[3], K. DO[1], N. TONI[2];
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Abstract: Schizophrenia (SZ) is a major psychiatric disease with high heterogeneity of symptoms, one of them being cognitive deficit, which cannot be improved by available treatments. It has been suggested that impaired adult hippocampal neurogenesis (AHN) may contribute to the cognitive deficit observed in patients. Indeed, hippocampal volume reduction is a robust observation in postmortem brains of patients and some studies also found a reduction of neural progenitors. Moreover, several animal models for SZ, such as DISC1 deletion and maternal immune activation, also show decreased AHN. Several factors are known to modulate AHN, including the redox state, inflammation, as well as parvalbumin expressing fast-spiking interneurons (PVI), which are key players in SZ pathophysiology.
The aim of this study is to investigate whether the interaction between these 3 main factors may lead to impaired AHN.
We took advantage of a mouse model for redox dysregulation, the GCLM KO mice, that show SZ related phenotype. These mice have 70% decreased glutathione, an important antioxidant, and increased oxidative stress in the brain throughout life, as well as decreased PVI in the ventral, but not dorsal, hippocampus, which was related with impaired behavior related to this specific region (Steullet et al., 2010). We investigated the proliferation rate of hippocampal

progenitors and newborn neurons survival in the dorsal versus ventral hippocampus of adult GCLM KO and WT mice, using BrdU incorporation assays.
Preliminary data show a dysregulation in the proliferation and survival of newborn neurons in the GCLM KO dentate gyrus. These results suggest that the oxidative stress level in the dentate gyrus of the GCLM KO mice may affect AHN.
Further investigation will bring light on the mechanism underlying the involvement of redox and immune dysregulation, as well as PVI, on neurogenesis impairments in SZ.

Disclosures: **B. Giangreco:** None. **D. Dwir:** None. **J. Kocher-Braissant:** None. **J. Cabungcal:** None. **S. Sultan:** None. **P. Steullet:** None. **E. Gebara:** None. **R. Chrast:** None. **K. Do:** None. **N. Toni:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.02/D16

Topic: A.02. Postnatal Neurogenesis

Support: ARC grant DP150104168

Title: Age-related changes in growth factor expression in the human subependymal zone: Relationship to cell proliferation and neuronal differentiation

Authors: *C. WEISSLEDER[1], S. J. FUNG[1], M. W. WONG[1], G. BARRY[2], K. L. DOUBLE[3], G. M. HALLIDAY[1], M. J. WEBSTER[4], C. SHANNON WEICKERT[1];
[1]Neurosci. Res. Australia, Sydney, Australia; [2]Garvan Inst. of Med. Res., Sydney, Australia; [3]Brain and Mind Res. Inst., Sydney, Australia; [4]Stanley Med. Res. Inst., Bethesda, MD

Abstract: Background: Adult neurogenesis in the human subependymal zone (SEZ, also subventricular zone) adjacent to the lateral ventricles declines during normal human aging. However, it is unknown how expression of growth factors related to neurogenesis change in the SEZ throughout adulthood or how altered transcript levels may affect cell proliferation and neuronal differentiation.
Methods: In this study, we used quantitative polymerase chain reaction to measure mRNA expression of growth factors [epidermal growth factor (EGF), transforming growth factor alpha (TGFα), fibroblast growth factor 2 (FGF2)] and receptors [EGF receptor (EGFR), Erb-B2 receptor tyrosine kinase 4 (ErbB4), FGF receptor 1 (FGFR1)] in the human SEZ throughout adulthood (n=50, 21-103 years). We further performed correlation analysis between growth factor-related transcripts and expression of neurogenic (cell proliferation, neuronal

differentiation) and glial cell markers (astrocytes, microglia, oligodendrocytes).
Results: EGFR and FGF2 mRNAs increased with age, while TGFα, EGF, ErbB4 and FGFR1 mRNAs were unchanged during aging. Cell proliferation and immature neuron marker mRNAs were positively correlated with TGFα and ErbB4 expression. Astrocyte and microglial cell marker mRNAs showed a positive correlation with EGF, FGF2 and FGFR1 transcript levels, whereas EGFR expression positively correlated with oligodendrocyte marker mRNA.
Conclusion: Our findings indicate that EGF and FGF family members do not become limited with age and may modulate neurogenesis and gliogenesis in the human SEZ. Additional studies are required to anatomically map the expression of these factors to cell types and determine their regulatory role along with other important trophic factors involved in adult neurogenesis across the human lifespan.

Disclosures: C. Weissleder: None. **S.J. Fung:** None. **M.W. Wong:** None. **G. Barry:** None. **K.L. Double:** None. **G.M. Halliday:** None. **M.J. Webster:** None. **C. Shannon Weickert:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.03/D17

Topic: A.02. Postnatal Neurogenesis

Title: Akt-mTOR pathway interactions control postnatal neurogenesis

Authors: *A. FOSTER, J. MERCURIO, M. FINGER, N. W. HARTMAN;
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Abstract: The Akt pathway is important for cellular growth, proliferation and differentiation. Akt activation has been proposed to increase cellular differentiation and protein translation by activation of the mammalian target of rapamycin (mTOR) pathway. It is unclear whether Akt and mTOR play roles independent of each other in NSC development. NSCs in the subventricular zone (SVZ) continually generate new daughter cells that migrate to the olfactory bulb (OB), differentiating into neurons. Here, we show that a constitutively active form of Akt resulted in a threefold increase in the number of newly born neurons in the OB. In contrast to driving mTOR alone, Akt activation did not result in apparent aberrant migration in the SVZ or OB. Akt activation resulted in increased dendritic length and complexity; however, total dendritic length was not rescued via mTORC1 inhibition. Activation of the Akt pathway was also observed to push NSCs into cell cycle progression and differentiation. Blockade of downstream mTORC1 targets inhibited differentiation, but did not alter the number of

proliferative cells in the SVZ. These data suggest that Akt controls NSC differentiation via mTOR, but may exert proliferative effects on NSCs independently.

Disclosures: **A. Foster:** None. **J. Mercurio:** None. **M. Finger:** None. **N.W. Hartman:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.04/D18

Topic: A.02. Postnatal Neurogenesis

Support: R25 NS065729

R01 NS095348

Title: The cytoplasmic FMRP interacting protein (CYFIP1) regulates neural stem cell proliferation and the maintenance of the neurogenic niche in the adult brain.

Authors: ***C. W. HABELA**[1], K.-J. YOON[1], G.-L. MING[2], H. SONG[2];
[1]Neurol., [2]Neurology, Inst. of Cell Engin. and the Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: The gene encoding cytoplasmic FMRP interacting protein (CYFIP1) is within the 15q11.2 copy number variants (CNVs), deletion and duplication of which have been linked to multiple neural developmental disorders including epilepsy, autism, intellectual disability and schizophrenia. There is increasing data to support it as an important protein in both normal and abnormal brain development. We previously showed that CYFIP1 is required for appropriate adherens junction formation in the neurogenic epithelium of the fetal brain of mice and loss of CYFIP1 function is associated with dysregulated embryonic cortical neurogenesis (Yoon et al. Cell Stem Cell 2014). However, it is not known whether CYFIP1 continues to facilitate the maintenance of the neurogenic niche and regulate neurogenesis in the postnatal period. The adult subventricular zone demonstrates continued postnatal neurogenesis in mammals. It is similar to the ventricular zone of development in that appropriate maintenance of its three dimensional structure is required for normal neurogenesis to occur. We hypothesized that CYFIP1 may continue to play a role in maintaining normal architecture in the SVZ neurogenic niche in the adult brain and that loss of its function would lead to dysregulation of neurogenesis here. We used both spatial and temporally controlled genetic knockdown of the *cyfip1* gene in a mouse model along with confocal microscopy to address this question. Loss of *cyfip1* in adult neural stem cells results in an increase in cell proliferation as well as loss of ependymal cells at the

ventricular surface. Our results suggest that CYFIP1 is required to maintain normal SVZ architecture throughout both pre- and post-natal development and can regulate neural stem cell behavior in the developing and mature brain. Dysregulation of CYFIP1 at either time-point may lead to neural dysfunction and its continued study is important for our understanding of neurologic disorders.
Grant support: R25 NS065729 and R01NS095348.

Disclosures: C.W. Habela: None. **K. Yoon:** None. **G. Ming:** None. **H. Song:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.05/D19

Topic: A.02. Postnatal Neurogenesis

Support: Medizinische Forschungskommission Universitaet Duesseldorf

Title: Inhibition of adult neurogenesis impairs the normal balance between multiple gaba and glutamate receptors in various cortical and subcortical brain regions

Authors: *C. HEROLD[1], J. DEITERSEN[1], K. AMUNTS[1,2], K. ZILLES[2,3];
[1]Heinrich Heine Univ. Duesseldorf, C. & O. Vogt-Institute of Brain Res., Duesseldorf, Germany; [2]Inst. of Neurosci. and Med. INM-1, Res. Ctr. Juelich, Juelich, Germany; [3]Dept. of Psychiatry, Psychotherapy and Psychosomatics, RWTH Aachen University, and JARA – Translational Brain Med., Aachen, Germany

Abstract: Impaired adult neurogenesis has been linked to a number of neurological diseases that are often accompanied by cognitive dysfunctions. However, for a lot of these functions, performance depends on the optimal stimulation of neurotransmitter receptors in the hippocampal-fronto-striatal system. Thus modulating adult neurogenesis may have impact on these networks and result in changes at the level of signaling molecules. Here, we analyzed binding site densities for glutamatergic AMPA, NMDA, kainate and $mGluR_{2/3}$ receptors, GABAergic $GABA_A$ (including benzodiazepine binding sites), $GABA_B$, and dopaminergic $D_{1/5}$ and $D_{2/3}$ receptors using quantitative *in vitro* receptor autoradiography in numerous cortical regions and basal ganglia of mice treated with temozolomide (TMZ) to impair adult neurogenesis in comparison to a saline control group. In the HF of TMZ treated mice we detected increased $GABA_B$ receptor densities in the dentate gyrus (DG) and fields of the Cornu ammonis (CA1 and CA3). Additionally, we observed decreased AMPA receptor densities exclusively in the molecular layer of DG, while NMDA receptor densities dropped in the

subgranular and the molecular layer of DG and in all layers of CA1 to CA3. No changes were observed in the hilar region. The prelimbic area (PL), dorsal infralimbic area (ILAd), dorsal and ventral agranular cingulate area (AcAd, AcAv), frontal area 2 (FR2) and gustatory area (GU) showed increased $GABA_B$ receptor levels. $GABA_A$ receptor densities were increased in PL, ILA, AcAd, AcAv and medial orbital area (MO/DP). While kainate receptor densities were increased in the ventrolateral and lateral orbital area (vLO and LO) and in ILA, $mGluR_{2/3}$ receptors showed decreased levels in FR2, GU, vLO, LO, dorsal and ventral agranular insular area (AId and AIv), and claustrum (CLA). NMDA receptor densities dropped in GU, AId, AIv, vLO and CLA. In the basal ganglia increased $GABA_B$ receptor densities were detected in the Ncl. accumbens (ACB), while $GABA_A$ receptor densities increased in the globus pallidus (GP). Decreased levels of $mGluR_{2/3}$ receptors were detected in ACB, while NMDA receptors additionally decreased in caudate/putamen (CP). A slight decrease of $D_{2/3}$ receptors was observed in ACB core and CP. None of the analyzed areas showed changes for BZ binding sites or in $D_{1/5}$ receptor densities. To our knowledge, this is the first study that reports neurochemical changes in the hippocampal-frontal-striatal network after inhibition of adult neurogenesis. We demonstrated that the inhibition of adult neurogenesis leads to impairments of the normal balance of the expression of multiple transmitter receptors in various cortical and subcortical regions.

Disclosures: **C. Herold:** None. **J. Deitersen:** None. **K. Amunts:** None. **K. Zilles:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

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Program#/Poster#: 494.06/D20

Topic: A.02. Postnatal Neurogenesis

Support: Bavarian Research Network “FORIPS”

Interdisciplinary Center for Clinical Research Friedrich-Alexander Universität Erlangen-Nürnberg

DFG (LI858/9-1)

Title: Sox11 target genes shape the neuronal cytoskeleton

Authors: J. VON WITTGENSTEIN[1], M.-T. WITTMANN[1], K. STEIB[2], K. DOBERAUER[2], I. SCHÄFFNER[1], E.-A. BALTA[1], B. HÄBERLE[1], J. BECKERS[2], M. IRMLER[2], F. ZHENG[1], C. ALZHEIMER[1], *D. C. LIE[1];

[1]Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; [2]Helmholtz Ctr. Munich German Res. Ctr. for Envrn. Hlth., Munich, Germany

Abstract: During adult neurogenesis adult-born neurons undergo a drastic change in morphology from proliferating precursor cells of variable shape to post-mitotic neurons with axon and a complex dendritic tree. While it has been shown that remodeling of microtubules is essential for the formation of axon and dendrites, it is not yet understood how the composition and stability of microtubules is regulated to enable integration of the newborn cell into an already existing network.
We have previously identified the transcription factor Sox11 as a key regulator of neuronal differentiation and fate determination during adult hippocampal neurogenesis. To get insight into the regulatory network of Sox11 we performed transcriptome analyses of in vitro differentiating neural stem cells. Strikingly, following KO of the SoxC group proteins Sox11&4, expression of many tubulin isoforms was dysregulated along with a number of microtubule-associated proteins, among them Doublecortin (DCX), MAP2, Spastin, and Stathmin1 (Stmn). Importantly, further biochemical analyses as well as in vivo gain- and loss-of-function experiments confirmed that SoxC proteins can directly activate the promoters of a subset of neuronal cytoskeleton-related proteins.
Our results indicate that SoxC proteins constitute a central hub in the transcriptional network of genes regulating microtubule composition during neuronal development. Future work is directed at the question whether SoxC proteins also regulate cytoskeletal remodeling in the context of neuronal plasticity.

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Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.07/D21

Topic: A.02. Postnatal Neurogenesis

Title: D-serine modulates hippocampal neurogenesis through dual CREB recruitment on the promoters of NMDA subunits and neurogenic genes

Authors: *S. BARBATI, L. LEONE, S. FUSCO, K. GIRONI, M. D' ASCENZO, C. GRASSI; Univ. Cattolica, Med. Sch., Rome, Italy

Abstract: Adult hippocampal neurogenesis has a critical role in brain plasticity, learning and memory. The co-agonist of the glutamate N-methyl-D-aspartate (NMDA) receptor, D-serine, has been recently involved in the modulation of adult hippocampal neurogenesis but the underlying molecular mechanisms have not been fully identified. We found that administration of D-serine (100 μM) to cultured neural stem cells (NSCs) increased their proliferation (+35% BrdU-positive cells compared to control, $p<0.05$) and accelerated their neuronal differentiation (+115% DCX-positive cells compared to control, $p<0.05$). To identify the molecular mechanisms responsible for these effects we performed quantitative real time PCR (qRT-PCR) experiments on adult hippocampal NSCs cultured under both proliferating and differentiating conditions. Exogenous administration of D-serine to the culture medium significantly increased the expression of the pro-proliferative gene Hes1 (+217%, $p<0.05$) in undifferentiated NSCs and of the determination gene Mash1 whose expression was upregulated (+50%, $p<0.05$) and temporally anticipated compared to control NSCs. Furthermore, qRT-PCR experiments performed on differentiating NSC extracts revealed an accelerated expression pattern of NMDA receptor subunits in D-serine treated NSCs consisting in earlier and increased expression of the NR1, NR2A and NR2B subunits (+300%, +260% and +75% respectively, $p<0.05$) after 24 h of D-serine administration. Interestingly, immunofluorescence data showed that exposure to D-serine increased the activation of the transcription factor CREB (pCREB) both in proliferative (+60%) and in differentiative conditions (+65%). Moreover, chromatin immunoprecipitation experiment revealed the recruitment of pCREB on the promoters of Hes1 and NMDA subunits and its higher binding in presence of D-serine. Finally, whole-cell patch-clamp recordings displayed a significant increase in NMDA receptor-mediated currents in NSCs cultured for 24-72 hours with 100 μM D-serine, supporting our hypothesis that D-serine accelerates neuronal maturation of NSCs. Collectively, our results suggest that D-serine positively modulates proliferation and neuronal differentiation of hippocampal NSCs by epigenetic mechanisms leading to an accelerated pro-neuronal gene and NMDA receptor subunit expression.

Disclosures: **S. Barbati:** None. **L. Leone:** None. **S. Fusco:** None. **K. Gironi:** None. **M. D' Ascenzo:** None. **C. Grassi:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

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Program#/Poster#: 494.08/D22

Topic: A.02. Postnatal Neurogenesis

Support: NSFC (31571043)

NSFC (81571212)

Title: Histone H3 Lysine 27 demethylase UTX regulates postnatal hippocampal neurogenesis

Authors: G. TANG, Y. ZENG, H. DU, S. DAI, Q. TANG, Z.-Q. TENG, *C. LIU;
The State Key Lab. of Stem cell and Reproductive Biol., Inst. of Zoology, Chinese Acad. of Sci., Beijing City, China

Abstract: Neurogenesis exists in specific regions of postnatal and adult brains, which is tightly controlled by various epigenetic regulators. Understanding the epigenetic regulatory mechanisms of neurogenesis is necessary for developing new strategies for brain repair. The X chromosome-encoded histone demethylase UTX (also known as KDM6A) establishes transcriptionally permissive chromatin through removal of repressive trimethylation of histone H3 lysine 27 (H3K27me3). However, whether H3K27Me3 demethylation affects neurogenesis has remained largely unknown. To investigate this, we conditionally inactivated H3K27Me3 demethylases Utx in postnatal neural stem cells. We found that deletion of UTX in postnatal hippocampal neural stem cells promoted neurogenesis both in vivo and in vitro. Furthermore, we demonstrated that UTX mediates postnatal neurogenesis in hippocampus through its H3K27 demethylase activity. Loss of demethylase activity by conditional knock-out UTX in neural stem cells resulted in higher H3K27Me3 intensity. Finally, we found that UTX promotes H3K27Me3 removal at a specific subset of genes involved in neural stem cells proliferation and differentiation. Thus, we have identified a critical role for the enzymatic activity of UTX in modulating neural-specific gene expression during hippocampal neurogenesis. # Correspondence should be addressed to:tengzq@ioz.ac.cn, liuchm@ioz.ac.cn

Disclosures: G. Tang: None. **Y. Zeng:** None. **H. Du:** None. **S. Dai:** None. **Q. Tang:** None. **Z. Teng:** None. **C. Liu:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: HKUST661111

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Title: Functional rols of Rho-GTPase-activating proteins in adult neurogenesis

Authors: ***Y. SU**[1,2,3], A. K. FU[1,2,3], L. MIN[1,2,3], J. P. IP[1,2,3], N. Y. IP[1,2,3];
[1]Div. of Life Sci., [2]Mol. Neurosci. Ctr., [3]State Key Lab. of Mol. Neurosci., The Hong Kong Univ. of Sci. and Technol., Hong Kong, China

Abstract: Continuous generation of neurons in the subgranular zone of the dentate gyrus in the hippocampus occurs throughout life. Its dysfunction may lead to neurological disorders such as autism, epilepsy, and schizophrenia. Rho GTPase-activating proteins (Rho-GAP) are a family of regulatory proteins that bind to activated Rho GTPases, stimulating their GTPase activity. While Rho-GAPs regulate neuronal differentiation, neurite outgrowth, and migration during CNS development, whether they are important for the regulation of adult neurogenesis remains largely unknown. Here, we report the role of the Rho-GAP α2-chimaerin in adult neurogenesis. The results show that α2-chimaerin-knockout mice exhibited impaired adult hippocampal neurogenesis. This effect was mediated through the regulation of neural progenitor cell proliferation, but not differentiation or maturation. Conditional knockout of α2-chimaerin in nestin-positive cells shifted the division mode of neural progenitors from asymmetric to symmetric division. Moreover, deficiency of α2-chimaerin attenuated the dendritic development of adult-born neurons in the dentate gyrus. Our findings reveal the functional roles of Rho GTPase-activating proteins in adult neurogenesis and provide a possible target for drug development for the treatment of neurological disorders.

Disclosures: **Y. Su:** None. **A.K. Fu:** None. **L. Min:** None. **J.P. Ip:** None. **N.Y. Ip:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant NS090926

NIH Grant NS089770

Title: A role for the signaling sphingolipid sphingosine-1-phosphate in the development of adult-born neurons

Authors: *A. DIANTONIO, C. YANG, J. WANG, Y. GU, S. GE;
Neurobio., Stony Brook Univ., Stony Brook, NY

Abstract: New neurons are continuously produced in the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus in adult mammals. In fact, up to 80% of dentate granule cells, the principal cells of the DG, are generated postnatally. Newborn neurons migrate within the DG, from the SGZ towards the molecular layer, where they eventually mature, form synapses, and integrate into existing circuits. There are many factors which influence neurogenesis, including the bioactive signaling sphingolipid sphingosine-1- phosphate (S1P). S1P acts as an extracellular ligand for the S1PR subfamily of G-protein- coupled receptors, and this signaling pathway affects cellular migration and neurite outgrowth. However, most of the studies on S1P's effects on neurogenesis have been conducted in vitro and focused on embryonic development. We are interested in the effects of S1P on the development of adult newborn neurons in vivo. Using RNA sequencing, we identified components of the S1P signaling pathway as potential factors involved in adult neurogenesis. To examine the potential of S1P to regulate adult neurogenesis, we quantified S1P levels in the DG via high-performance liquid chromatography-mass spectrometry and confirmed that the ligand was indeed present in this brain region in adult animals. Additionally, multiple behavioral treatments known to enhance neurogenesis led to decreased levels of S1P in the DG. We also used immunohistochemistry to determine that two of the five known S1PRs - specifically, S1P1 and S1P2 - are expressed in adult-born neurons. Using retroviral vectors to manipulate S1PR expression, we have found that cellular migration and dendritic arborization are affected by altered S1PR signaling.

Disclosures: **A. Diantonio:** None. **C. Yang:** None. **J. Wang:** None. **Y. Gu:** None. **S. Ge:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

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Program#/Poster#: 494.11/D25

Topic: A.02. Postnatal Neurogenesis

Support: MH087473

Title: A mouse model for probing the significance of regulated intramembrane proteolysis of Neuregulin 1 in neural development

Authors: *A. JONE, P. RAJEBHOSALE, L. W. ROLE, D. A. TALMAG;
Neurobio. and Behavior, Stony Brook Univ., Centereach, NY

Abstract: Neuregulin 1 (Nrg1) is a family of versatile signaling proteins extensively involved in neural development and synaptic plasticity. One of the neuron-specific isoforms type III Nrg1 is critical for neuronal survival, neural fate determination, receptor trafficking, axon myelination, and synaptic transmission. Genetic deletion of Type III Nrg1 in mice results in deficits in synaptic plasticity and behavioral deficits involving working memory and sensorimotor gating. Upon ErbB4 binding or neuronal depolarization, Type III Nrg1 undergoes regulated intramembranous proteolysis (RIP), mediated in part by γ-secretase cleavage, to generate a carboxyl-terminal fragment. This soluble intracellular domain (ICD) of Type III Nrg1 is capable of translocating to the nucleus, where it possesses strong transcriptional transactivation properties. *In vitro*, the valine residue at position 321 is necessary for proper RIP of Type III Nrg1 and appropriate dendritic arborization. Interestingly, a single-nucleotide polymorphism that has been associated with psychosis and schizophrenia in a human population in Costa Rica results in the substitution of a leucine for this valine residue.
We generated a valine-321-leucine (V321L) mouse line and asked whether this point mutation affects Nrg1 RIP and disrupts developmental events *in vivo*. Through subcellular fractionation, we show that both full-length Nrg1 and the ICD fragment are enriched in the membrane fraction extracted from V321L homozygous mouse brain lysate compared to that of wild-type, suggesting that Nrg1 RIP is impaired by the V321L mutation. Because the ICD has been previously demonstrated to regulate neural stem cell proliferation and fate specification *in vitro*, we wondered whether neurogenesis is affected by disruptions in Nrg1 RIP *in vivo*. Ongoing studies are investigating the effect of this substitution on hippocampal neurogenesis in young adult mice. Preliminary results indicate that mice with the V321L mutation have reduced cell proliferation and generation of newborn neurons in the dentate gyrus compared to wild-type littermates.

Disclosures: **A. Jone:** None. **P. Rajebhosale:** None. **L.W. Role:** None. **D.A. Talmag:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.12/D26

Topic: A.02. Postnatal Neurogenesis

Title: Conditional deletion of cannabinoid CB1 receptor alters neural precursor proliferation and affects neurogenesis-dependent behavior and synaptic plasticity

Authors: *T. ZIMMERMANN[1], M. MAROSO[2], S. LUDEWIG[3], I. SOLTESZ[2], M. KORTE[3], B. LUTZ[1], J. LESCHIK[1];
[1]Inst. of Physiological Chem., Univ. Med. Ctr. Mainz, Mainz, Germany; [2]Dept. of Neurosurg., Stanford Univ., Stanford, CA; [3]Zoological Institute, Div. Cell. Neurobio., TU Braunschweig, Braunschweig, Germany

Abstract: Adult born neurons in the subgranular zone (SGZ) of the dentate gyrus are continuously generated and incorporated in the hippocampal circuitry, yet the molecular mechanism of this process remain unclear. Adult neural stem cells contain a functional endocannabinoid system. Endocannabinoids are endogenous lipids, which can bind to the presynaptic cannabinoid 1 (CB1) receptor, thereby inhibit neurotransmitter release and activate different intracellular signaling cascades [1]. It has been shown that loss of CB1 receptor signaling inhibits neural precursor proliferation and decreases astroglial differentiation *in vitro* and *in vivo* [2]. However, it is not known, if this phenomenon is regulated through a direct or an indirect mechanism of CB1 receptor signaling.
To address this question, we generated the triple-transgenic nestin-CreERT2/R26R-YFP/CB1flox/flox mouse line. Tamoxifen (TAM) injections induced deletion of the CB1 receptor and expression of yellow fluorescent protein (YFP) specifically in adult nestin-expressing stem cells and their progeny. To assess which neurogenic stages were affected by elimination of CB1, YFP SGZ cells were assigned to categories based on expression of immunohistochemistry markers.
We found that neural stem cell-specific deletion of the CB1 receptor led to a reduced number of total YFP cells in the dentate gyrus four weeks after TAM injection and to a decrease in neural stem cell proliferation, as assessed by BrdU staining. Animals lacking a functional CB1 receptor in newborn neurons displayed a decrease in spatial memory function in the spatial object recognition test and an increase in behavioral despair in the forced swim test. Anxiety-like behavior in the light dark test and spontaneous activity in the open field were not affected. Field excitatory postsynaptic potentials (fEPSPs) were recorded in the CA1 region by stimulating Schaffer collateral axons of area CA3. TAM treated triple-transgenic Nestin-CreERT2/R26R-YFP/CB1flox/flox mice displayed a long-term potentiation (LTP) curve that is different to that of littermate controls.
The present study shows that the proliferation and survival of newborn neurons critically depends on the activation of the CB1 receptor, reflecting the importance of the functional connectivity and the involvement on the behavioral level.

References:

[1] Katona I, Freund TF (2012) Annu Rev Neurosci 35:529-58
[2] Aguado T, Palazuelos J, Monory K, Stella N, Cravatt B, Lutz B, Marsicano G, Kokaia Z, Guzman M, Galve-Roperh I (2006) J Neurosci 26(5):1551-61

Disclosures: T. Zimmermann: None. **M. Maroso:** None. **S. Ludewig:** None. **I. Soltesz:** None. **M. Korte:** None. **B. Lutz:** None. **J. Leschik:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

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Program#/Poster#: 494.13/D27

Topic: A.02. Postnatal Neurogenesis

Support: Fond Léon Frederic

FRIA

FNRS

Title: A crucial role for Cdk1 in postnatal hippocampal neurogenesis

Authors: *Q. MARLIER[1], R. VANDENBOSCH[1], N. CARON[2], S. VERTENEUIL[1], F. JIBASSIA[1], P. KALDIS[3], L. N'GUYEN[1], B. MALGRANGE[1];
[1]Developmental Neurobio. Unit, Univ. of Liège, Giga-Neurosciences, Liege, Belgium; [2]Laboratoire&management, Affichem, Toulouse, France; [3]Inst. of Mol. and Cell Biol., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Age-related neurological disorders, including stroke and neurodegenerative diseases are becoming a major health care problem in many countries as average life expectancy is increasing. One appealing therapeutic strategy to treat neurological disorders would consist of recruiting endogenous neural precursor cells (NPCs) to replace the lost neurons. NPCs are present in two specific areas of the postnatal brain, the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles. These NPCs give rise to neurons throughout life, a phenomenon termed postnatal neurogenesis. A prerequisite for the development of new therapeutic strategies involving postnatal NPCs is a better understanding of their molecular regulation. In this context, both extrinsic factors and intrinsic factors have been identified. For instance, the family of cell cycle regulators cyclin-dependent kinases (Cdks) are key regulators of postnatal neurogenesis since genetic invalidation of interphase Cdk2 or Cdk6 leads to a decrease of NPCs proliferation. Here, we investigated the role of Cdk1, an essential Cdk for M-phase in several tissues, in postnatal neurogenesis. Towards that purpose, we crossed mice bearing a conditional Cdk1 allele ($Cdk1^{lox}$) with mice expressing a tamoxifen-inducible form of the Cre-recombinase under the control of the Sox2 promoter (Sox2CreER), allowing us to specifically delete Cdk1 in Sox2+ NPCs in the postnatal brain. Following Cdk1 loss, our preliminary results show a acute decrease in the percentage of recombinated proliferating Sox2+ cells as well as a decrease in the absolute number of Sox2+ cells in the DG. Interestingly, we also observed an increased number of DCX+ progenitors cells and a long-term depletion of the pool of Sox2+ in the absence of Cdk1. The number of neurons seems to decrease in the same

time. Altogether, these data suggest a crucial role for Cdk1 in postnatal neurogenesis, but further investigations are required to identify its precise requirement in this process.

Disclosures: **Q. Marlier:** None. **R. Vandenbosch:** None. **N. Caron:** None. **S. Verteneuil:** None. **F. Jibassia:** None. **P. Kaldis:** None. **L. N'guyen:** None. **B. Malgrange:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: Fondecyt Nº1150933

Title: Frizzled-1 regulates fate determination of neural progenitor cells in the adult hippocampus

Authors: ***L. VARELA-NALLAR**, S. B. ARREDONDO, M. D. MARDONES;
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Abstract: The generation of new granule neurons in the adult hippocampus is controlled by the Wnt signaling pathway, which regulates different stages of neurogenesis including proliferation and differentiation of neural progenitor cells. We have determined that the Wnt receptor Frizzled-1 (FZD1) is expressed in neural stem cells (NSCs), transient-amplifying progenitors and immature neurons in the adult dentate gyrus. Here, we investigated the potential role of this receptor on proliferation and cell fate determination of progenitor cells. To knockdown the expression of FZD1 in proliferating cells in vivo, retroviruses expressing FZD1-targeting shRNA (shFZD1) or control shRNA (shC) were stereotaxically injected into the dentate gyrus of 2-month-old mice. FZD1 knockdown did not induce changes in proliferation of NSCs or neural progenitor cells as assessed by staining for Ki67 and specific markers. In agreement, no changes in the pool of tranduced NSCs and neural progenitors were observed between shC and shFZD1-injected mice 1 week after retroviral injection. However, there was a strong decrease in the percentage of FZD1-deficient cells expressing the immature neuronal marker doublecortin. Four weeks after retroviral injection there was a significant decrease in the percentage of shFZD1-transduced cells that became granule neurons, and concomitantly with this reduced generation of new neurons there was an increase in the percentage of FZD1-deficient cells that became astrocytes. In N2a cells and cultured adult hippocampal progenitors isolated from mouse brain, FZD1 knockdown reduced the canonical Wnt/β-catenin signaling pathway and the expression of Wnt target genes, including proneural transcription factors. Our results indicate that FZD1

receptor regulates cell fate commitment, and suggest that this effect is mediated by the activation of the canonical Wnt signaling pathway.

Disclosures: **L. Varela-Nallar:** None. **S.B. Arredondo:** None. **M.D. Mardones:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.15/D29

Topic: A.02. Postnatal Neurogenesis

Support: DFG

Title: Role of PTEN phosphorylation in brain development

Authors: ***J. LEDDEROSE**[1], S. GÖGEL[1], W. BINTIG[1], F. FURNARI[2], B. J. EICKHOLT[1]; [1]Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Ludiwig Inst. for Cancer Res., UCSD, San Diego, CA

Abstract: Phosphatase and tensin homolog located on chromosome 10 (PTEN) was originally characterized as a tumor suppressor that can inhibit proliferation, migration, cell growth and apoptosis in a number of different cells. PTEN is also highly expressed in neurons and recent work indicates that de-regulation of PTEN affects important neuronal functions in the developing nervous system, which have been attributed to its role in controlling neurogenesis, neurite outgrowth, synaptogenesis, and synaptic plasticity. Human germline PTEN mutations or conditional deletions of PTEN in mice have provided insights into fundamental links between PTEN deregulation and neurological disorders such as macrocephaly, ataxia, seizures, mental retardation and autism. The regulation of PTEN activity and function crucially involves phosphorylation of a cluster of serine and threonine residues in the PTEN C-terminus. Within this cluster, PTEN can be phosphorylated at T366 by Glycogen Synthase Kinase 3 (GSK3), which has been suggested to regulate activity and PTEN protein turnover. Since PTEN antagonises and functions as a key upstream regulator of PI3K/GSK3 signalling, the fact that it is phosphorylated and regulated also by GSK3 itself, could indicate feedback regulation within this signalling cascade. Currently, little is known concerning neuronal functions of PTEN T366 phosphorylation. To determine the contribution of PTEN T366 to neuronal development and/or function, we analyzed mice in which this phosphorylation site was inactivated as a result of the introduction of a germline point mutation ($PTEN^{T366A}$). Whilst homozygous $PTEN^{T366A/T366A}$ mice, hereafter referred to as $PTEN^{T366A}$ mice, are viable and fertile, subtle differences in cortical lamination were observed. Thus, we will present our data that investigates cortical neurogenesis

and post-neurogenesis processes in different cortical layers of $PTEN^{T366A}$ mice, with an emphasis on the cell-type specificity and fine organization of dendritic arbors. Our results highlight specific roles of PTEN-T366 phosphorylation in generating anatomical and functional synaptic connections, possibly affecting sensory processing and higher cognitive function.

Disclosures: J. Ledderose: None. **S. Gögel:** None. **W. Bintig:** None. **F. Furnari:** None. **B.J. Eickholt:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.16/D30

Topic: A.02. Postnatal Neurogenesis

Title: Plexin-A1 is crucial for developmental neuronal death in cerebellum

Authors: *K. YUKAWA, T. ITO, M. HOSSAIN, T. NEGISHI, K. YOSHIDA;
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Abstract: In the developing brain, microglia actively promotes apoptotic neuronal death. The microglial DAP12 immunoreceptor and CD11b integrin cooperatively induce neuronal apoptosis by controlling the production of superoxide ions. Our coimmunoprecipitation experiment has disclosed the association of DAP12 with the plexin-A1 semaphorin receptor and TREM2 in postnatal microglia. Thus, microglial plexin-A1 and DAP12 may cooperatively work to induce developmental neuronal death. We have reported that the interaction of Sema6D on dying neurons and microglial plexin-A1 is crucial for the execution of neuronal apoptosis in the developing hippocampus. To examine if plexin-A1 is also crucial for neuronal death in the developing cerebellum, we analyzed neuronal apoptosis, microglial distribution, semaphorin expression and superoxide ions production in wild-type (WT) and plexin-A1-deficient cerebella from postnatal day 1 (PND1) to postnatal day 14 (PND14). In the developing cerebellum, some of apoptotic neurons were localized in contact with microglia. There was a significant decrease of apoptotic cells in the plexin-A1-deficient cerebellum at postnatal day 3 and 4 (PND3 and 4) as compared with wild-type (WT). The generation of superoxide ions was mostly confined to Iba1-positive microglia. The superoxide ions-producing cells were significantly decreased in plexin-A1-deficient cerebella reflecting the dependency of superoxide ions generation on plexin-A1 signaling. The expression level of Sema6D, one of plexin-A1 ligands was significantly higher in cerebella at PND4 as compared with other developmental stages. Furthermore, activated caspase-3-positive neurons exhibited the increase of Sema6D expression in cerebella at PND4. Thus, Sema6D expressed by dying neurons may signal to microglia through the binding to

microglial plexin-A1, which facilitates the generation of superoxide ions and neuronal apoptosis in the developing cerebellum. Since activated caspase-3-positive neurons are distinct from calbindin-positive Purkinje cells, we are currently trying to identify the cell type which executes plexin-A1-dependent apoptosis in the cerebella at PND4.

Disclosures: K. Yukawa: None. **T. Ito:** None. **M. Hossain:** None. **T. Negishi:** None. **K. Yoshida:** None.

Poster

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Topic: A.02. Postnatal Neurogenesis

Support: VI on ALS/FTD, DZNE

Title: Specific functions of redox signalling in maintaining neural stem cells in hippocampal neurogenesis

Authors: *V. S. ADUSUMILLI[1,2], T. L. WALKER[1,2], A. E. RÜNKER[1,2], R. W. OVERALL[1,2], G. KEMPERMANN[1,2];
[1]CENTER FOR REGENERATIVE THERAPIES DRESDEN (CRTD), Dresden, Germany;
[2]Deutsches Zentrum für Neurodegenerative Erkrankengen (DZNE), Dresden, Germany

Abstract: Resident neural stem cells (NSCs) of the subventricular zone (SVZ) and the dentate gyrus of the hippocampus (DG) generate new neurons, which integrate into existing circuits throughout life ("adult neurogenesis"). Though the broad scheme of adult neurogenesis is similar in both the neurogenic zones, there are important differences. Importantly, whereas wheel running is a potent pro-proliferative cue in the DG, it has no effect on SVZ neurogenesis. We hypothesized that a central metabolic pathway, which is uniquely regulated in the hippocampal NSCs, serves as the responsive checkpoint in response to the pro-neurogenic stimuli. A comparison of the expression profiles of Nestin-positive precursor cells from the two zones revealed redox regulation to be one such differentially regulated pathway. All the NSCs of the DG have high endogenous levels of reactive oxygen species (ROS), the readout of redox regulation, compared to the surrounding niche cells. All neurosphere-forming cells were found within a population with highest ROS content (top 16 %). Furthermore, neurosphere-forming frequency significantly correlated to the ROS content, as did the size of the spheres. ROS content also was a discriminating marker for the heterogeneous metabolic states of NSCs and allowed classifying them into subgroups, which differed in various cell biological properties. The

proportion of NSCs with the highest ROS content (termed ROS-Hi cluster) was significantly greater in the DG. The response to physical activity primarily occurred in this subgroup of NSCs. Reducing the subgroup of ROS-Hi NSCs did reduce constitutive neurogenesis but completely abolished the pro-neurogenic response of exercise

Disclosures: V.S. Adusumilli: None. **T.L. Walker:** None. **A.E. Rünker:** None. **R.W. Overall:** None. **G. Kempermann:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.18/D32

Topic: A.02. Postnatal Neurogenesis

Title: Single cell transcriptome analysis of adult neurogenesis during aging

Authors: *Y. ZHU[1,2], D. JIMENEZ-CYRUS[3], D. BERG[1,2], M. BONAGUIDI[6], G.-L. MING[1,2,3,4,5], H. SONG[1,2,3,4];
[1]Inst. for Cell Engin., [2]Dept. of Neurol., [3]Cell. and Mol. Med. Grad. Program, [4]The Solomon H. Snyder Dept. of Neurosci., [5]Dept. of Psychiatry and Behavioral Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [6]Eli and Edythe Broad CIRM Ctr. for Regenerative Med. and Stem Cell Res., USC, Los Angeles, CA

Abstract: During aging, the regenerative capacity of stem cells in mammalian tissue declines. Specifically, the process of new neuron generation from neural stem cells (NSC) is progressively less efficient during aging. This is associated with a gradual increase in stem cell quiescence and a shift toward symmetric divisions and self-renewal. Characterizing the molecular dynamics that occur in individual stem cells over the lifespan is important not only to understand the biology of aging, but also to explore the potential of stem cells for regeneration. Traditionally, characterization of NSCs using marker-defined cell population studies inevitably included cells at different stages of fate determination and activation, making it difficult to dissect the age-related changes that affect the properties of NSCs at specific stages of neurogenesis. Here we utilized the high resolution and high throughput technology of single cell transcriptome analysis to comprehensively characterize the molecular states underlying fate determination in NSCs from young and aged mice. Using a line of mice expressing nuclear CFP under the control of a Nestin promoter (Nes-CFP), we isolated hippocampal Nes-CFP positive cells from two week-old, and one, two and four month-old mice and profiled the transcriptome. In older mice, we observed more NSCs exhibiting molecular signatures indicative of a quiescent state. In early neural progenitor cells, we detected higher levels of cell cycle gene expression, potentially to

compensate for the lower rates of stem cell activation. Gene network analysis indicates a key regulatory node that could orchestrate the quiescent nature of adult NSCs. Our work established a holistic road map of NSC gene expression dynamics during aging. Further work on functional characterization will validate and consolidate these findings.

Disclosures: Y. Zhu: None. **D. Jimenez-Cyrus:** None. **D. Berg:** None. **M. Bonaguidi:** None. **G. Ming:** None. **H. Song:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.19/D33

Topic: A.02. Postnatal Neurogenesis

Support: HRF-201512-012

Title: EAAC1 gene deletion reduces adult hippocampal neurogenesis after transient cerebral ischemia

Authors: *B. CHOI[1], S. WON[2], M. SOHN[3], S. SUH[1];
[1]Dept. of Physiol., Hallym University, Col. of Med., Chuncheon, Korea, Republic of; [2]Dept. of Neurol., Univ. of California San Francisco and Veterans Affairs Med. Ctr., San Francisco, CA; [3]Dept. of Nursing, Inha Univ., Incheon, Korea, Republic of

Abstract: The excitatory amino acid carrier-1 (*EAAC1*) is one of the most prevalent glutamate transporters expressed in neurons. *EAAC1* expression is detected in the dendrites and somata of all neurons in the hippocampus. Since extracellular glutamate clearance in the brain is predominantly performed by the glial glutamate transporters, such as GLAST and GLT-1, glutamate clearance by *EAAC1* is negligible in the brain. Instead, *EAAC1* predominantly performs cysteine transport, which is required for the glutathione synthesis, to boost neuronal antioxidant function. Our previous study showed that genetic deletion of *EAAC1* was found to exhibit increased susceptibility to neuronal oxidative stress after ischemia due to reduced antioxidative function. There are presently no studies investigating the role of *EAAC1* on hippocampal neurogenesis. Therefore, we have investigated the role of *EAAC1* on hippocampal neurogenesis using genetically modified young (3-5 month old) and aged (11-15 months old) mice after ischemia. Adult CD1 wild-type (WT) or *EAAC1*$^{-/-}$ mice were subjected to 30 minutes of bilateral common carotid artery occlusion. BrdU was intraperitoneally injected 2 times per day for 4 consecutive days starting 3 days after ischemia. Neurogenesis was evaluated using BrdU, Ki67 and doublecortin (DCX) immunostaining 7 or 30 days after ischemia. The number of BrdU,

Ki67 and DCX positive cells between WT and *EAAC1*$^{-/-}$ mice was similar at 1 week under the normal physiological conditions. However, *EAAC1*$^{-/-}$ mice showed a reduced survival rate of newly generated neurons 4 week later in both young and aged mice. The number of BrdU, Ki67 and DCX positive cells was increased 1 week after ischemia both in young and aged mice of either WT or *EAAC1*$^{-/-}$. However, the number of BrdU, Ki67 and DCX positive cells was significantly lower in young *EAAC1*$^{-/-}$ mice compared to young WT mice. In addition, *EAAC1*$^{-/-}$ mice showed reduced survival rate of newly generated neurons at 4 weeks after ischemia in both young and aged mice. Newly generated neuron survival was lower in *EAAC1*$^{-/-}$ mice than WT mice. The present study demonstrates that cysteine uptake by *EAAC1* is important for newly-generated neuron survival in the adult brain under physiological as well as pathological conditions. Therefore, this study suggests that *EAAC1* has an essential role for modulating hippocampal neurogenesis.

Disclosures: **B. Choi:** None. **S. Won:** None. **M. Sohn:** None. **S. Suh:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

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Topic: A.02. Postnatal Neurogenesis

Title: Integration and functional role of different sub-populations of granule cells in the adult olfactory bulb.

Authors: *D. HARDY, S. MALVAUT, V. BRETON-PROVENCHER, A. SAGHATELYAN; CRIUSMQ, Québec, QC, Canada

Abstract: In the adult brain, the olfactory bulb is continuously supplied with new neurons that mostly differentiate into granule cells (GC). Different subtypes of adult-born GC have been identified based on immunohistochemical markers and localization in the granule cell layer, but their maturational profile, as well as their role in the bulbar network functioning and odor behaviour remain completely unclear. It is also unknown if structural and functional differences exist in the same sub-population of GC born during early postnatal life and in adulthood. Using viral injections, transgenic mice, confocal imaging, whole-cell patch-clamp recordings, pharmacogenetic and behavioral tests, we analyzed the structural and functional properties of different sub-populations of GC born during early postnatal period and adulthood with particular emphasis on calretinin-expressing newborn cells (CR+). Our morphological analysis performed at several time points after generation of early-born and adult-born neurons revealed that CR+ GC display similar maturational pattern as compared to CR- cells. Patch-clamp recordings from

early-born and adult-born CR+ GC showed similar frequency and amplitude of spontaneous inhibitory currents. In contrast, our results suggest that adult-born CR+ GC may receive less frequent excitatory inputs as compared to early-born CR+ neurons. Our analysis of early gene expression combined with anti-calretinin immunohistochemistry suggests that CR+ GC may be involved in the long-term odor associative memory. Altogether, our data highlight the role of CR+ GC in the OB and are important for understanding the role of specific sub-populations of interneurons in the bulbar network functioning and odor behavior.

Disclosures: D. Hardy: None. **S. Malvaut:** None. **V. Breton-Provencher:** None. **A. Saghatelyan:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

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Program#/Poster#: 494.21/E1

Topic: A.02. Postnatal Neurogenesis

Support: Swedish Research Council

Title: Let-7 regulates adult neurogenesis by activating autophagy

Authors: *R. PETRI, K. PIRCS, M. JÖNSSON, M. ÅKERBLOM, P. L. BRATTÅS, T. KLÜSSENDORF, J. JAKOBSSON;
Lund Univ., Lund, Sweden

Abstract: In the adult rodent brain, neural stem cells (NSCs) reside in the subventricular zone (SVZ) where they continuously give rise to neuroblasts that migrate along the rostral migratory stream (RMS) into the olfactory bulb (OB). There they differentiate into mature neurons and integrate into the existing neuronal circuitry.
Recently microRNAs (miRNAs) were shown to regulate important processes during adult neurogenesis such as cell fate specification and integration. miRNAs are small, non-coding, single stranded RNAs that regulate mRNA activity by associating with a protein called Argonaute2 (AGO2).
In this study, we injected lentiviral vectors encoding an AGO2-GFP fusion protein into the RMS. After eight weeks we dissected the OB, and conducted AGO2-RIPseq to isolate active miRNAs from new-born neurons. We identified several novel miRNAs that are enriched in new-born neurons and found that the let-7 family is the predominant miRNA in these cells. To study the role of let-7 in new-born neurons we inhibited let-7 function by injecting a miRNA-sponge construct (LV.let-7sp.GFP) into the RMS. As control we injected a vector encoding GFP

(LV.GFP). We immunohistochemically analysed OBs after 2, 4 and 8 weeks. In control animals, we found GFP-positive cells in all layers of the OB at any time examined. In LV.let-7sp.GFP injected animals, however, the cells reached the OB but failed to integrate into its different layers.
To identify potential let-7 targets in the OB that could be involved in the observed phenotype, we conducted further AGO2-RIP experiments followed by mRNA sequencing.
We found Slc7a5 to be highly enriched in RIP samples and to be a direct target of let-7. Slc7a5 is part of the amino acid sensing pathway, which has previously been shown to be regulated by let-7 to induce neuronal autophagy.
In line with this we found an accumulation of Sequestosome-1 (p62) in LV.let-7sp.GFP- injected animals, suggesting that loss of let-7 induces a block of autophagy in new-born neurons .
We then injected LV.let-7sp.GFP constructs alone or together with BECN and TFEB overexpressing lentiviral vectors, respectively, into the RMS. TFEB and BECN are both factors known to promote autophagy. After four weeks, we analysed OBs immunohistochemically for autophagic markers and confirmed a reinstatement of autophagy in co-injected animals. Moreover, significantly more GFP-positive cells were integrating into the different layers of the OB when autophagy was reinstated, compared to LV.let-7sp.GFP injected animals.
Taken together our study provides a novel and important link between miRNA regulation, autophagy and adult neurogenesis.

Disclosures: **R. Petri:** None. **K. Pircs:** None. **M. Jönsson:** None. **M. Åkerblom:** None. **P.L. Brattås:** None. **T. Klüssendorf:** None. **J. Jakobsson:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: FAPESP Grant 2013/12928-8

Title: XRN2 and PAPD4 provide an extra miRNA regulation level in neurons

Authors: *V. PASCHON[1], D. L. CAMPOS[2], F. F. CORREIA[2], N. D. R. NOGUEIRA[2], V. A. DA SILVA[2], A. H. KIHARA[2];
[2]Nucleo de Cognição e Sistemas Complexos, [1]Univ. Federal Do ABC, São Bernardo Do Campo, Brazil

Abstract: MiRNA is a hotspot in various fundamental processes, such as neurodevelopment, adaptation, and disease. These small non-coding RNAs are important regulators of gene expression at the post-transcriptional level, acting on the mRNA, decreasing the translation rate. The action of miRNAs on target genes depends on intracellular concentration, which in turn reflects the balance of biosynthesis and degradation. The aim of this project was to characterize the expression and distribution of genes involved in degradation and stability of miRNAs, XRN2 and PAPD4, during rat spinal cord development, neuronal activation by physics exercise and after injury. Quantitative results were determined using real-time PCR and western blotting, whose data were subjected to analysis of variance (ANOVA) followed by Tukey's test, with a significance level of 1-5%. Specific antibodies were used to determine the qualitative analyzes by immunofluorescence. For neunoal activation, rats were trainned following the protocol (Quirie, Hervieu et al. 2012). XRN2 and PAPD4 were highly expressed in the beginning of spinal cord development. PAPD4 was 527% higher in E21 in comparison with P60 ($P<0.05$) and XRN2 was 263% higher in E21 in comparison with P60 ($P<0.05$). Both gens showed distinct sub cellular localization by immunohistochemistry, accumulating in nuclei and cytoplasm during development or only in the nuclei of mature neurons. Combined analyzes using anti-XRN2 and -PAPD4 with anti-Ki67 and -glutamine synthetase (GS) revealed that miRNA regulating proteins were poorly expressed in progenitor cells as well as glial cells. Moreover, double-labeling experiments with choline acetyltransferase (ChAT), calretinin (CR) and calbindin (CB), specific spinal cord neuronal markers, showed that XRN2 and PAPD4 are expressed by motor neurons (ChAT-positive neurons) and inter neurons labeled with CR and CB. Those results indicate that XRN2 and PAPD4 provide an extra level of regulation for miRNA activity in immature and developed neurons comparing with glial cells. The neuronal activation by physical exercise did not change XRN2 labeling but increased the number of cells expressing PAPD4, while both protein levels were up-regulated. Finally, the spinal cord injury provides a large number of TUNEL-positive cells after 24-hours whatever fell off then accumulated XRN2 and PAPD4 showing potent anti-apoptotic roles. In summary, this work brings knowledge to increase the understanding in the field of miRNA regulation during development, neuronal function and after injury. Financial support: FAPESP.

Disclosures: **V. Paschon:** None. **D.L. Campos:** None. **F.F. Correia:** None. **N.D.R. Nogueira:** None. **V.A. da Silva:** None. **A.H. Kihara:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: NINDS P01NS045260-01

NINDS R01NS057128

NIMH R15MH101703

Title: Calpain-1 deletion results in reduced survival of newborn cells in the adult mouse brain.

Authors: *J. SEINFELD, C. ABUYO, J. LE, X. BI, M. BAUDRY;
Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Recent studies estimate that hundreds of new neurons are added to the adult human brain every day, and several reports have proposed that neurogenesis participates in cognition, including learning and memory. Previous studies have suggested that the calcium-dependent proteases, calpains, participate in cell division, migration and survival. In particular, our laboratory has established that one of the calpain isoforms in the brain, calpain-1, is necessary for the survival of newly born cerebellar granule cells (CGCs), as calpain-1 knock-out (KO) mice exhibit enhanced apoptosis during the postnatal period and decreased number of CGCs in adult cerebellum. In the present study we analyzed proliferation and survival of newly born cells in the dentate gyrus (DG), the subventricular zone (SVZ), the rostral migratory stream (RMS), and the olfactory bulbs (OB) in calpain-1 KO and wild-type (WT) mice. Cell proliferation was decreased in the DG of calpain-1 KO mice by 25%, as measured by Ki67 expression with immunohistochemistry (IHC). Survival of newly-generated cells in the OB was decreased by 47% in calpain-1 KO mice 30 days after bromodeoxyuridine (BrdU) administration (150 mg/kg per day for three days). On the other hand, comparable numbers of BrdU-positive cells were found in the RMS of calpain-1 KO and WT mice. These results are consistent with the hypothesis that calpain-1 is necessary for neuronal survival in mouse brain, not only during the postnatal period but also in adult brain. We are currently investigating the molecular mechanisms by which calpain-1 activation supports the survival of newly born cells in the brain. Preliminary results with primary cultures of fibroblasts derived from calpain-1 KO and WT mice suggest that calpain-1 also regulates migration. Further studies are needed to better define the roles of calpain-1 and calpain-2 in proliferation, migration and survival of newly-born neuronal precursor cells in the brain and the functional consequences of deletion/mutation of calpain-1.

Disclosures: **J. Seinfeld:** None. **C. Abuyo:** None. **J. Le:** None. **X. Bi:** None. **M. Baudry:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.24/E4

Topic: A.02. Postnatal Neurogenesis

Support: Japan Society for the Promotion of Science Research Fellowship for Young Scientists

Title: Role of neural stem cells in the sensory circumventricular organs of adult mouse

Authors: *E. FURUBE[1], M. MORITA[2], S. MIYATA[1];
[1]Applied Biol., Kyoto Inst. of Technol., Kyoto, Japan; [2]Develop. of Biol., Kobe Univ., Hyogo, Japan

Abstract: The adult brain contains neural stem cells (NSCs) that are self-renewing and generate neurons, astrocytes and oligodendrocytes. The sensory circumventricular organs (sCVOs), including the organum vasculosum of the lamina terminalis (OVLT), subfornical organ (SFO), and area postrema (AP), are shown to have NSCs, but the characterization and functional significance of NSCs in these regions are completely unknown. Employing Nestin-Cre/CAG-CAT-EGFP mice, we showed that there were many enhanced green fluorescent protein (EGFP)-expressing astrocyte-like cells and tanycyte-like ependymal cells in the sCVOs. Moreover, EGFP-expressing astrocyte-like cells expressed glial fibrillary acidic protein (GFAP) in the SFO, OVLT and AP and tanycyte-like ependymal cells often expressed GFAP only in the SFO. It was shown that these astrocyte-like cells and tanycyte-like ependymal cells showed bromodeoxyurigine (BrdU) incorporation into their nuclei. Lineage-tracing fate determination experiments using Nestin-Cre/CAG-CAT-EGFP mice and BrdU immunohistochemistry provided evidence that NSC originated within the sCVOs provided neural and glial cells to their inside and their neighboring regions such as the medial preoptic area, ventral hippocampal commissure, the nucleus of solitary tract, and 12N. Furthermore, we also found expression of temperature, acid and capsaicin receptor transient receptor potential vanilloid 1 (TRPV1) and TRPV1-depend activation of JAK-signal transducer and activator of transcription 3 in NSCs of the sCVOs. In addition to that, NF-κB signaling was induced in NSCs of the sCVOs via the activation of bacteria lipopolysaccharide receptor Toll-like receptor 4.These results suggest that astrocyte-/tanycyte-like NSCs in the sCVOs act as sensors to detect blood-derived information in addition to the new generation of neurons and glial cells.

Disclosures: **E. Furube:** Other; Japan Society for the Promotion of Science Research Fellowship for Young Scientists. **M. Morita:** None. **S. Miyata:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

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Topic: A.02. Postnatal Neurogenesis

Support: NSERC Discovery grant (JSS)

NSERC PGSD

Killam Doctoral Funding

Title: Early survival and delayed death of developmentally-born dentate gyrus neurons

Authors: *S. P. CAHILL, R. Q. YU, E. TODOROVA, D. GREEN, J. S. SNYDER;
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Abstract: Much effort has been spent trying to understand the function of adult-generated hippocampal neurons. At the cellular level, adult-generated neurons progress through defined developmental stages that are characterized by specific patterns of synapse formation, marker expression, physiological function and cell death. Less is known about the developmental timecourse of neurons born during the perinatal period. To address this question we injected rats with the mitotic marker BrdU at postnatal day 6, to label neurons born at the peak of dentate gyrus development. We then counted and characterized BrdU+ cells at 1hr, 1d, 3d, 1w, 2w, 3w, 4w and 8w timepoints. Unlike adult-born neurons, which undergo significant cell death during the first 4 weeks after mitosis, we observed no loss of developmentally-born cells after the 1w timepoint. Developmentally-born neurons upregulated NeuN and downregulated DCX comparably to what has been observed in adult-born neurons. To assess functional integration of BrdU+ neurons, we examined expression of the immediate-early genes c-fos and zif268 (rats explored a novel environment). Similar to what has been observed in adult-born neurons, there was an early peak in zif268 expression. However, peak zif268 expression occurred when neurons were 2w old, which is 1w earlier than adult-born cells and consistent with evidence that developmentally-born neurons mature faster than adult-born neurons. Exploration-induced c-fos expression was present in a smaller proportion of neurons, did not show an early peak, and plateaued at the 4w timepoint. To investigate whether developmentally-born neurons continue to survive throughout adulthood we injected additional groups of rats with BrdU at postnatal day 6 and quantified surviving BrdU+ cells at 2 months and 6 months. In contrast to the stable survival between 1w-8w, we observed a 17% loss of BrdU+ cells between 2-6months. Developmentally-born neurons expressed caspase3 when rats were 2 months old, providing additional evidence that these neurons underwent cell death. Collectively, our findings indicate that developmentally-born and adult-born neurons differ markedly in their patterns of survival: during stages of

neuronal immaturity, adult-born neurons die but developmentally-born neurons are stable; when neurons have reached maturity, adult-born neurons are stable and developmentally-born neurons die. By identifying temporal patterns by which developmentally-born persist throughout the lifespan, these findings may be relevant for understanding the dynamic nature of hippocampal memory.

Disclosures: **S.P. Cahill:** None. **R.Q. Yu:** None. **E. Todorova:** None. **D. Green:** None. **J.S. Snyder:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.26/E6

Topic: A.01. Neurogenesis and Gliogenesis

Support: BFU2011-27326

RD12/0019/0024

PROMETEO II/2014/014

Title: Study of neurovascular niche in dentate gyrus in Fgfr1 mutant mice

Authors: **A. POMBERO**[1], R. GARCIA-LOPEZ[2], *S. MARTINEZ[2];
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Abstract: The concept of neurogenic niche in the brain proposes a functional unit between the precursor cells and their local microenvironment. Neural stem cells in the neurogenic areas proliferate in groups close to the perivascular space where their self-renewal and differentiation is regulated. The central nervous system pericytes have been recognized as an indispensable component of the neurovascular unit (NVU). In the hippocampus, this is important for providing an optimal microenvironment for neural proliferation. Hippocampal neurovascular regulatory system include both diffusible signals and direct contact with endothelial and pericytes, which are a source of diffusible neurotrophic signals such as VEGF and FGFs, that affect neural precursors. One of the key growth factors that regulate postnatal neurogenesis in hippocampus is fibroblast growth factor-2 (FGF-2, also called basic FGF). FGF2 is expressed by endothelial and periendothelial cells (pericytes) and FGF2 deficiency produces a decrease of neural proliferation in the dentate gyrus. Interestingly, hippocampal neural precursors express Fgfr1, the major receptor for FGF2 and its absence leads to a decrease in neurogenesis accompanied by a severe

impairment of long-term potentiation and memory consolidation. Previous studies suggest that FGFs act directly on hippocampal stem cells. Since FGF-2 is a potential regulator in neurogenesis and angiogenesis crosstalk, we propose to describe the precise role of FGF signal in NVU development in the hippocampus by studying whether the neurovascular niche is altered in FgfR1 mutant mice. In order to address it we focused on pericytes and endothelial cells to detect vascular abnormalities during development, and subsequent anomalies in neuro-epithelial specification, migration and differentiation of hippocampal granular cells and neurogenic niche in the dentate gyrus. This work was supported by: FEDER BFU2011-27326, Red TERCEL RD12/0019/0024, Generalitat Valenciana: PROMETEO II/2014/014 and Walk on Project Association (WOP)

Disclosures: **A. Pombero:** None. **R. Garcia-Lopez:** None. **S. Martinez:** None.

Poster

494. Molecular Mechanisms of Adult Neurogenesis

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 494.27/E7

Topic: A.02. Postnatal Neurogenesis

Support: Russian Ministry of Education and Science Grant 11.G34.31.0071

RFBR Grant 15-29-01305

RSCF Grant 16-15-00294

Title: WM-CLICK, a new method for 3D detection and representation of dividing cells in the whole brain

Authors: ***A. LAZUTKIN**[1,2,3,4], S. SHUVAEV[1,3], I. DORONIN[1], N. BARYKINA[1], E. AMELCHENKO[4], K. ANOKHIN[2,5], G. ENIKOLOPOV[1,3,4];
[1]Brai Stem Cell Lab., MIPT, Moscow, Russian Federation; [2]Lab. of Systemogenesis of Behavior, P.K.Anokhin Inst. of Normal Physiol., Moscow, Russian Federation; [3]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [4]Dept. of Anesthesiol., Stony Brook Univ., Stony Brook, NY; [5]NBICS, Dept. of Neurobio., Kurchatov Inst., Moscow, Russian Federation

Abstract: Ability to visualize dividing stem cells in the whole animal brain would facilitate analysis of neurogenesis dynamics and increase its accuracy, and may reveal hidden patterns of cell division and neurogenesis. Here we describe new histological techniques for 3D visualization and quantitation of proliferating cells in the whole brain of developing and adult mice. Populations of dividing cells were labeled with 5-ethynyl-2'-deoxyuridine (EdU) and

detected using whole-mount click-reaction with fluorescent azide (WM-CLICK). Our method allowed staining entire adult mouse brain and hippocampi and visualizing patterns of cell division using confocal microscopy, light-sheet microscopy, and two-photon tomography. WM-CLICK is compatible with simultaneous detection of dividing cells and stem cells revealed by whole-mount immunohistochemistry and is well suited for automatic counting of stem and proliferating cells in acquired 3D images. These new approaches were validated by conventional methods for detection and quantitation of dividing stem cells. We applied our techniques for visualization and 3D comparison of division and migration patterns in whole perinatal and adult brain. Our data demonstrate the utility of the WM-CLICK methods for quantitative and descriptive analysis of neurogenesis.

Disclosures: **A. Lazutkin:** None. **S. Shuvaev:** None. **I. Doronin:** None. **N. Barykina:** None. **E. Amelchenko:** None. **K. Anokhin:** None. **G. Enikolopov:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.01/E8

Topic: A.03. Stem Cells and Reprogramming

Support: Human Spare Parts program of Tekes

Finnish Cultural Foundation

Doctoral Programme in Biomedicine and Biotechnology

The Paulo Foundation

Title: Two-photon polymerized microstructures for guiding *In vitro* human neuron growth

Authors: ***T. JOKI**[1], S. TURUNEN[2], L. YLÄ-OUTINEN[1], M. KELLOMÄKI[2], S. NARKILAHTI[1];
[1]NeuroGroup, Biomeditech / Univ. of Tampere, Tampere, Finland; [2]Biomaterials and Tissue Engin. Group, BioMediTech/ Tampere Univ. of Technol., Tampere, Finland

Abstract: Introduction
Neural cells in vivo have polarized cell morphologies and form tissue with highly organized anatomical structures and functional circuits. These properties are altered due to cell death related to trauma or deficit, leading a decrease in patient's ability to function. In humans the

regeneration capacity of nervous system is very limited. Tissue engineering, e.g. transplantation therapy with either biomaterial scaffold or cells alone or their combination, could in future offer new treatments to enhance functionality. In defected area the scaffold can act as an artificial extra cellular matrix (ECM), supporting transplanted and/or host cells thus enhancing regeneration. Cell growth guidance along the anatomical structure of tissue is desirable for enhancing integration of transplant and regeneration of functional neural circuit. For guidance, mechanical and chemical cues can be used to enhance tissue mimicking organized growth. The aim of this work was to study the effect of topographical cues on growth of human neural cells in vitro using 3D microstructure platform.
Materials and methods
Microstructures for cell cultures were manufactured using two-photon polymerization (2PP). Neuronal cells were produced from human pluripotent stem cells (hPSCs) using a neurosphere differentiation method. Human embryonic stem cell (hESC) line Regea 08/023 derived neurons were cultured on the microstructures up to four weeks. Cultures were analyzed in time points 1, 2 and 4 weeks. Cell viability was studied using fluorescent LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells. Cell growth was analyzed using scanning electron microscopy (SEM). Cell phenotype was analyzed using immunocytochemical analysis against neuron specific proteins, MAP-2 and β Tubulin III.
Results
Microstructures were manufactured with 2PP successfully and repeatably. Cell viability was good during 4 weeks follow-up time. Both neuronal processes and cell soma migrated along the microstructures according to SEM analysis. Cultured cells had neuronal phenotype confirmed by MAP-2 and β Tubulin III protein expression by immunocytochemistry.
Conclusions
Produced microstructures provided favorable microenvironments for growth of human pluripotent stem cell derived neurons. Cells were able to migrate along topographical cues and form 3D networks utilizing the microstructures. The experimental set-up was successful and offers new ideas for the field of in vitro 3D culturing.

Disclosures: **T. Joki:** None. **S. Turunen:** None. **L. Ylä-Outinen:** None. **M. Kellomäki:** None. **S. Narkilahti:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.02/E9

Topic: A.03. Stem Cells and Reprogramming

Title: Lineage specific effects of the Sonic Hedgehog pathway on the differentiation of human embryonic stem cells

Authors: *H. GU[1,2], Y. GALAT[4,2], D. O. WALTERHOUSE[4,2,5], V. GALAT[4,2], P. IANNACCONE[4,2,3];
[1]Developmental Biol. Program, Ann & Robert H. Lurie Children'S Hosp. of Chica, Chicago, IL; [2]Pediatrics, [3]Pathology, Northwestern Univ. Feinberg Sch. of Med., Chicago, IL;
[4]Developmental Biol. Program, Ann & Robert H. Lurie Children Hosp. of Chicago, Chicago, IL; [5]Cancer Biol. & Epigenomics, Ann & Robert H. Lurie Children's Hosp. of Chicago, Chicago, IL

Abstract: GLI1 is one of three GLI family transcription factors that mediate the Sonic Hedgehog (SHH) signal transduction pathway. The pathway plays an important role in normal development and cell differentiation. GLI1 is also a human oncogene with gene targets that sustain proliferation, inhibit apoptosis, promote angiogenesis, and promote tumor cell migration. GLI1 is highly expressed in embryonic stem cells (ESCs), neural stem cells (NSCs), and mesenchymal stem cells (MSCs). Over-expression of SHH and GLI1 in stem cells enhances production of neural progenitor and dopaminergic neurons. GLI1 can bind the promotor of Nanog and activate its transcription for regulating self-renewal of NSCs. However, Nanog binds GLI proteins in ESCs and represses GLI1-mediated transcriptional activation. The effects and mechanisms of action of the SHH pathway on differentiation of human ESCs are incompletely understood. Here, we studied the role of the SHH signal transduction pathway in differentiation of human ESCs. For the spontaneous differentiation, we used embryoid body (EB) assay. Small molecules and genetic modification were used to manipulate GLI1 activity. Gene expression and lineage differentiation were evaluated by qPCR, western blot, immunocytochemistry, and biochemical approaches. The GLI1 antagonist, GANT61, promoted mesodermal and endodermal differentiation, but decreased ectodermal differentiation in human ESCs. The roles of the SHH pathway directing the differentiation of human ESCs were investigated by comparing the results of high-throughout screening assays, including mass spectrometry and chromatin immunoprecipitation sequencing (ChIP-Seq) with published sequencing databases. These results indicate that SHH pathway has lineage specific effects on the differentiation of embryonic stem cells.

Disclosures: H. Gu: None. **Y. Galat:** None. **D.O. Walterhouse:** None. **V. Galat:** None. **P. Iannaccone:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.03/E10

Topic: A.03. Stem Cells and Reprogramming

Title: Maturation-dependent acquisition of anti-apoptotic brakes in human induced pluripotent stem cell-derived neurons

Authors: *K. KLEINSIMLINGHAUS, J. JATHO-GRÖGER, J. LADEWIG, P. KOCH; Inst. of Reconstructive Neurobio., Bonn, Germany

Abstract: Neurons represent a highly specialized cell population of the central and peripheral nervous system responsible for communicating information throughout the body. They are born during embryonic development and persist throughout the entire life span of an individual. During development, neurons are produced in excess numbers, which are massively reduced during the establishment of mature neuronal circuitries. While cell death pathways are freely active in immature neurons, mature neurons develop elaborate survival strategies to protect themselves against stress and apoptosis. We set out to decipher the pathways associated with increased resistance of mature human neurons to stress and apoptosis using human pluripotent stem cell (iPSC)-derived neuronal cultures. We show that during the time course of maturation, iPSC-derived neurons become increasingly resistant to several types of cellular stressors. This goes along with a failure to activate cellular caspases. Comparative gene expression profiling of immature and mature neurons identified several pathways differentially regulated in both cell types, which are implemented in cell survival, stress response and autophagy. We got interested in a small heat shock protein, which was strongly upregulated in mature neurons compared to their immature counterparts. Small heat shock proteins might be of particular interest, because the classical heat shock response is attenuated in mature neurons. In line with this we could show that Hsp70 and Hsp27 are downregulated in mature neuronal cultures compared to immature neurons. To investigate the specific contribution of the identified protein we generated knockout cell lines using CRISPR/Cas9-mediated gene editing. Indeed, disruption of the genes increased the susceptibility of knockout neurons to some, but not all stress conditions. Our experiments shed new light on pathways associated with increased resistance of mature human neurons to stress and provide a platform to investigate novel mechanistic principles in neuroprotection and neurodegeneration.

Disclosures: K. Kleinsimlinghaus: None. **J. Jatho-Gröger:** None. **J. Ladewig:** None. **P. Koch:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.04/E11

Topic: A.01. Neurogenesis and Gliogenesis

Support: project no. LQ1605 from the National Program of Sustainability II (MEYS CR)

project FNUSA-ICRC no. CZ.1.05/1.1.00/02.0123 (OP VaVpI)

Title: Advancing development of stem cell derived neuron/glia co-culture to allow for their optimal physiological testing

Authors: *M. CARNA, V. POZO DEVOTO, V. LACOVICH, K. TEXLOVA, G. STOKIN; Translational Neurosci. and Aging Res. Group, Intl. Clin. Res. Ctr. FNUSA-ICRC, Brno, Czech Republic

Abstract: To date, different protocols have been used to differentiate hESC into neuronal and glial lineages, however, their maturation processes and functionality assays vary significantly among the studies. Moreover, the majority of protocols used to differentiate hESC into astrocytes include serum in the differentiation media, which leads to the production of activated astrocytes since the very beginning of the maturation process. This finding, together with lack of critically addressing interdependency of co-cultures, precludes physiological testing of the interaction between neurons, astrocytes and oligodendrocytes and their functionality.In this study, we have designed a protocol allowing for Neural Stem Cells (NSCs) (H9-derived) to be directed to nearly equivalent proportion of neurons, astrocytes and oligodendrocytes in serum free conditions. The medium was designed to develop brain-like environment to test for their interactions and functionality. More specifically, this protocol allows for simple, fast and efficient derivation of neurons, astrocytes and oligodendrocytes from a single batch of NSCs, which can be sorted into any physiological or pathological combination. We find that this newly designed co-culture system significantly improved both the astrocyte features and adequate support to neuronal activity in culture. These results indicate that the proposed platform can be used as a powerful tool to study neurobiological impact of glial cell on neural differentiation and development and promote different approach to study human neurological diseases in vitro. Further experiments with different types of stem cells will be needed to confirm our preliminary findings.

Disclosures: **M. Carna:** None. **V. Pozo Devoto:** None. **V. Lacovich:** None. **K. Texlova:** None. **G. Stokin:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.05/E12

Topic: A.01. Neurogenesis and Gliogenesis

Title: Assessing population diversity and transcriptome dynamics during *In vitro* differentiation of human GABAergic neurons

Authors: *J. L. CLOSE[1], Z. YAO[1], B. LEVI[1], J. MILLER[1], T. BAKKEN[1], V. MENON[1], A. WALL[3], A. KROSTAG[4], E. THOMSEN[3], J. TING[1], J. GRIMLEY[5], S. RAMANATHAN[6], E. LEIN[2];
[2]Human Cell Types, [1]Allen Inst. For Brain Sci., Seattle, WA; [3]Human Cell Types, [4]Allen Inst. for Brain Sci., Seattle, WA; [5]Universal Cells, Seattle, WA; [6]Harvard Univ., Cambridge, MA

Abstract: Inhibitory interneurons shape cortical processing through diverse patterns of connectivity, firing pattern, and neurochemical identity. These cells have been implicated in human neurodevelopmental and psychiatric disorders, and multiple protocols have described methods to generate these cells from hESCs and iPSCs in vitro for both research and therapeutic purposes. To assess the diversity of interneuron progenitors and neurons in culture, we developed a protocol to generate them from hESCs. We sorted progenitors from neurons using the DCX-Citrine hESC reporter line, RNAseq was performed at the subpopulation and single-cell level and transcriptomic differences between populations were analyzed over the course of differentiation. We find that the cells generated using this protocol reliably differentiate into MGE-like progenitors and GABAergic neurons, and that many of the genes known to play a role in this process are expressed. When the gene expression of cells generated in vitro were compared to migrating interneurons present in fetal brains, we found that in vitro-derived interneurons present at day 54 of differentiation were most similar to fetal interneurons present at 100 dpc. Multiple distinct populations of progenitors and neurons were present at each timepoint, allowing us to characterize the dynamics of cell type production in this system, as well as identify novel genes involved in cortical interneuron differentiation. We discovered that genes implicated in human diseases, such as epilepsy, autism and schizophrenia, are expressed as SST^+ cortical interneurons differentiate and become functional.

Disclosures: **J.L. Close:** None. **Z. Yao:** None. **B. Levi:** None. **J. Miller:** None. **T. Bakken:** None. **V. Menon:** None. **A. Wall:** None. **A. Krostag:** None. **E. Thomsen:** None. **J. Ting:** None. **J. Grimley:** None. **S. Ramanathan:** None. **E. Lein:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.06/E13

Topic: A.03. Stem Cells and Reprogramming

Support: Ministry of Innovation, Science and Research of the State of North Rhine-Westphalia

Title: Modeling malformations of cortical development in hiPSC-derived cortical organoids

Authors: *V. IEFREMOVA, G. MANIKAKIS, A. JABALI, R. WILKENS, K. WEYNANS, P. KOCH, J. LADEWIG;
Inst. of Reconstructive Neurobio., Bonn, Germany

Abstract: The development of the human cortex requires a precise choreography of progenitor proliferation, neurogenesis and neuronal migration, which can be disrupted in malformations of cortical development (MCDs). In the past, most studies on MCDs were performed using mouse models. Critical structural differences between human and mice might, however, necessitate the use of additional model systems. In this context, pluripotent stem cell (PSC)-derived three-dimensional (3D) cerebral organoids, which faithfully recapitulate certain aspect of human brain development in vitro, have emerged as an attractive alternative. Here we used cortical organoids derived from human induced pluripotent stem cells (iPSCs) to address pathophysiological changes associated with Miller-Dieker Syndrome (MDS), a severe form of MCD caused by a haploinsufficiency on chromosome 17p13.3 involving the genes *LIS1* and *YWHAE* (coding for 14.3.3ε). Both proteins, LIS1 and 14.3.3ε, are components of an intracellular multiprotein complex including NDEL1, which is essential for the regulation of cytoplasmic dynein, centrosomal protein localization and microtubule dynamics. When applying our cortical organoid model to iPSCs derived from patients with MDS, we found that typical pathological hallmarks of the disease can be recapitulated in vitro. In particular, we observed that organoids from MDS patients show a significant reduction in size, disruption of cortical microtubule, switch in spindle orientation of ventral radial glia cells and early neurogenesis. Phenotypic changes could be rescued by applying CRISPR/Cas9 mediated genome editing of either LIS1 or 14-3-3ε suggesting a role of both, LIS1 and 14.3.3ε, in early cortical progenitor expansion. These data indicate that iPSC derived 3D cortical organoids serve as a promising system to model complex MCD in vitro.

Disclosures: V. Iefremova: None. **G. Manikakis:** None. **A. Jabali:** None. **R. Wilkens:** None. **K. Weynans:** None. **P. Koch:** None. **J. Ladewig:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.07/E14

Topic: A.03. Stem Cells and Reprogramming

Support: Hannah's Hope Fund

Title: Novel generation methods for difficult giant axonal neuropathy (GAN) hiPS-derived neuronal cells allow studies of mitochondrial function and transport in mutant axons

Authors: P. MANOS[1], C. BJORNNSSON[2], S. LOTZ[2], A. ANNELING[2], *A. MESSER[2]; [1]StemCulture, Renssalaer, NY; [2]Regenerative Res. Fdn, Neural Stem Cell Inst., Rensselaer, NY

Abstract: Giant Axonal Neuropathy (GAN) is a rare pediatric neurodegeneration caused by mutations in the protein gigaxonin. This E3 ubiquitin ligase adaptor protein enables orderly turnover of intermediate filaments in neurons and vimentin filaments in fibroblasts. Neurons that fail to correctly process filaments form large neurofilament and peripherin aggregates, creating giant axons that become increasingly dysfunctional. Effects are most critical in motor neurons, leading to extreme weakness for both locomotion and breathing. Death occurs within two decades for the most severe cases. Although a human clinical trial of AAV9 intrathecal gene replacement has recently begun, a wide range of cells will remain uncorrected even the most effective gene therapy. Drug treatments that can reduce the vulnerability of cells to the stress of accumulating protein remain an important aspect of a combinatorial therapeutic strategy.
In order to screen candidate drugs, neurons generated from patient cells, particularly the most severe cases such as GAN, are very useful. Human induced pluripotent stem cells (hiPSCs) from GAN patients with missense mutations have been generated; however, severe homozygous deletion mutations also show large vimentin aggregates when fibroblasts are grown from biopsies. These donor cells show poor proliferation and reprogramming capacity using standard procedures. We therefore developed a novel culture protocol utilizing a continuous supply of FGF2 and EGF released from StemBeads®. StemBeads® are micrcoencapsulated proteins in a biodegradable polymer allowing the continuous release of proteins. StemBeads® significantly enhanced proliferation, total cell number, and increased capacity to reprogram and yielded higher efficiency. Further improvements in culture protocols for production of multiple types of neurons from homozygous deletion and missense cell lines has enabled us to follow defects in axons to test pharmacological interventions.
hiPSCs differentiated into cortical and motor neurons were stained with JC-1 to reveal mitochondrial dynamics and activity. Three-dimensional images collected on a multiphoton microscope were analyzed to track the movement of mitochondria along axonal processes,

followed by immunolabeling to confirm distribution of cytoskeletal proteins affected by GAN. This approach to use continuous growth factor exposure via StemBeads® to mimic in vivo conditions for optimal cell reprogramming and neuronal differentiation of hiPSCs, while allowing quantitative monitoring of mitochondrial transport and function, should be applicable to a wide range of neurodegenerative diseases.

Disclosures: **P. Manos:** A. Employment/Salary (full or part-time): StemCultures. **C. Bjornnsson:** None. **S. Lotz:** None. **A. Anneling:** None. **A. Messer:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.08/DP01 (Dynamic Poster)

Topic: A.03. Stem Cells and Reprogramming

Title: Systems level analysis of cellular diversity and neuronal activity in long-term cultures of human cerebral organoids

Authors: ***G. QUADRATO**[1,2], T. NGUYEN[1], E. MACOSKO[3,2], J. SHERWOOD[1,2], N. MARIA[1], Y. H. ZHANG[1], S. MCCARROLL[3,2], Z. WILLIAMS[3], P. ARLOTTA[1,2]; [1]SCRB, Harvard Univ., Cambridge, MA; [2]Broad Inst. of Harvard and MIT, Cambridge, MA; [3]Harvard Med. Sch., Boston, MA

Abstract: Genomic sequencing has rapidly expanded our understanding of the polymorphisms underlying complex neuropsychiatric disorders. However, we still have a limited understanding of the cellular phenotypes induced by these risk-associated alleles. 3D Cerebral organoids represent an emerging model that meets many of the criteria for an optimal *in vitro* system to phenotype specific mutations. However, current organoids appear to only represent early timepoints in brain development, and whether they can be used to phenotype the functional effects of psychiatric disease risk alleles on neuronal development and network behavior has not been fully explored. We have implemented a modified version of the culturing protocol developed by Lancaster et al.[1] to enable efficient long-term cultures, and have achieved healthy growth of cerebral organoids for over nine months. We have characterized a timecourse of generation of different brain structures and cell types within one to nine month old organoids by immunohistochemistry. This data shows that organoids develop by producing neural progenitors followed by neurogenesis. At later stages of differentiation, following a developmentally-correct temporal sequence, we observed astrogliogenesis and synaptogenesis paralleled by formation of active neuronal circuits. To understand the exact cellular composition of a cerebral organoid, we

used high-throughput single cell RNA sequencing via droplet sequencing[2] to profile a large number of cells (60,000 cells) across 19 six month old organoids. Analysis shows that organoids display a high degree of cellular diversity, including expression of markers of distinct subtypes of progenitors, glial cells, and class-specific markers of excitatory and inhibitory cortical neurons. We also identified cell-type-specific expression of autism and schizophrenia-linked genes, further validating the use of cerebral organoids to model complex neuropsychiatric disorders. Together, our data demonstrate that aspects of human brain development and circuit-level changes in physiological and pathological conditions can be modeled using long-term cultures of cerebral organoids.

1. Lancaster, MA et al. (2013) Cerebral organoids model human brain development and microcephaly. Nature
2. Macosko, EZ et al. (2015) Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. Cell

Disclosures: **G. Quadrato:** None. **T. Nguyen:** None. **E. Macosko:** None. **J. Sherwood:** None. **N. Maria:** None. **Y.H. Zhang:** None. **S. Mccarroll:** None. **Z. Williams:** None. **P. Arlotta:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.09/E15

Topic: A.03. Stem Cells and Reprogramming

Support: NIMH BRAINS Award (R01MH107800)

California Institute of Regenerative Medicine (CIRM)

MQ Fellow Award

Donald E. and Delia B. Baxter Foundation Award

Title: Modeling human interneuron migration and integration with a novel 3D forebrain culture approach

Authors: F. BIREY[1], J. ANDERSEN[1], C. D. MAKINSON[2], J. HUGUENARD[2], *S. P. PASCA[1];
[1]Psychiatry & Behavioral Sci., [2]Neurol. & Neurolog. Sci., Stanford Univ., Palo Alto, CA

Abstract: Progress in dissecting the molecular programs underlying human brain development and the subsequent emergence of network activity has been remarkably slow, stalling advances in understanding neuropsychiatric disorders. This is partly due to the lack of direct access to brain cells from patients, the slow translation of findings from rodent models, and the unavailability of functionally relevant in vitro models. Here, we developed a novel tridimensional (3D) differentiation method of human pluripotent stem cells (iPSC, ES) to generate neural spheroids resembling either the laminated neocortex (cerebral cortical spheroids) or the ventral telencephalon (subpallial spheroids), which includes cortical interneurons. Using labeled spheroids, we engineer two-region human forebrain structures, and employed state-of-the-art live imaging, electrophysiological recordings and calcium imaging techniques, to explore complex cell-to-cell interactions in the developing telencephalon and in the context of neurodevelopmental disease. Our results provide novel insights into the unique features of human cortical interneurons in the developing cortex.

Disclosures: F. Birey: None. **J. Andersen:** None. **C.D. Makinson:** None. **J. Huguenard:** None. **S.P. Pasca:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.10/E16

Topic: A.01. Neurogenesis and Gliogenesis

Support: Olle Engqvist foundation

The Crafoord Foundation

Olle and Edla Johansson Foundation

NanoLund

Gtreta and Johan Kocks Fund

Claes Groschisnky Fund

Title: Use of low-density, uncompressed nanofiber electrospun scaffolds for the culture of three-dimensional electrical active human neuronal networks

Authors: *U. ENGLUND JOHANSSON[1], A. JAKOBSSON[2], M. OTTOSSON[2], M. ZALIS[2], D. O´CARROLL[3], F. JOHANSSON[3];
[1]Div. of Ophthalmology, [2]Clin. Sciences, Lund, Div. of Ophthalmology, [3]Biology, Div. Zoophysiology, Lund Univ., Lund, Sweden

Abstract: For the majority of CNS disorders which leads to neuronal loss and impaired function, no treatment or cure exists. We hypothesize that clinical translation of promising experimental therapies may be more efficient if current 2D *in vitro* models are complemented with 3D *in vitro* models mimicking the native milieu. Although the generally accepted importance of the extracellular matrix (ECM), during nervous system development and in the homeostasis and function of the adult nervous system, this knowledge still need to be translated and its impact explored in *in vitro* neural cell-based systems. Hence, we here explored how a 3D physical culture environment effects the phenotypic differentiation of human neural progenitor cells (HNPCs) as compared to a traditional 2D culture system. Randomly oriented, 3D, sub-micron fiber networks (thickness: 200 µm, fiber diameter: 350-700 nm) of biodegradable polycaprolactone (PCL) was fabricated using a custom built Focused, Low-density, Uncompressed nanoFiber (FLUF) electrospinning setup. A mitogen-expanded multipotent HNPC line was used. At 20 days of culture the survival, neurogenic potential, neurite extension and functional development was studied using immunocytochemical-, biochemical- and electrophysiological techniques, and compared to controls cultured at 2D glass surfaces. Fluorescence-, confocal- and scanning electron microscopy were used. HNPC cultured in the 3D meshes showed excellent survival, potential to physically integrate in all three dimensions of the mesh and extension of long neurites. Importantly, HNPC demonstrated the capacity to mature into functional neurons as revealed by electrophysiological recordings. Notably, in 3D scaffolds *in vivo*-resembling intermixed neuronal- and glial cell network were formed, whereas in parallel 2D cultures a neuronal cell layer grew separated from an underlying glial cell layer. Encouragingly, our results show the potential use of electrospun, 3D, PCL nanofibrous scaffolds for long-term cultivation of human brain stem cells with the potential to form electrophysiological functioning neurons. Hence, 3D cell-scaffolds may be very useful in further developmental- as well as therapeutical studies on the nervous system.

Disclosures: U. Englund Johansson: None. **A. Jakobsson:** None. **M. Ottosson:** None. **M. Zalis:** None. **D. O´Carroll:** None. **F. Johansson:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.11/E17

Topic: A.03. Stem Cells and Reprogramming

Title: BrainPhys™ Neuronal Medium supports the electrical activities of neurons derived from human pluripotent stem cells and primary CNS tissues in long-term cultures

Authors: C. K. H. MAK[1], *V. M. LEE[1], L. H. CHEW[1], K. MCCORMACK[1], S. LLOYD-BURTON[1], A. C. EAVES[1,2], T. E. THOMAS[1], S. A. LOUIS[1];
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Abstract: Action potential firing and synaptic activity are fundamental properties of neurons in the brain. Bardy et al. (PNAS, 2015) have recently reported that Neurobasal™ Medium and DMEM/F-12 support neuron survival but suppress their synaptic activities in culture. To solve this problem, we have developed BrainPhys™ Neuronal Medium (BrainPhys™), based on the formulation published by Bardy et al., to support growth and synaptic function of neurons in long-term cultures. Here we describe the effect of BrainPhys™ and DMEM/F-12 based media on the development of neuronal electrical activity of human pluripotent stem cell (hPSC)-derived and primary E18 rat cortical neurons in 3 and 6 weeks cultures, respectively. For hPSC cultures, neural progenitor cells derived from either induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs) were differentiated in BrainPhys™ or DMEM/F-12 (control) with supplements, and cultured for 3 weeks. We performed half-medium changes every 3 - 4 days and measured the neuronal electrical activity twice a week using the multielectrode array (MEA) system. Our data showed that the mean firing rate of iPSC- and ESC-derived neurons (n = 1; 32 electrodes) in BrainPhys™ increased from <0.09 Hz on day 12, to 0.38 Hz and 0.17 Hz by day 25, respectively. In contrast, the mean firing rate of neurons in DMEM/F-12 remained low (<0.06 Hz) over the same 3-week period. For experiments using primary tissues, E18 rat cortical cells were plated in Neurobasal™ Medium with NeuroCult™ SM1 Neuronal Supplement (SM1). After 5 days, cultures were either transitioned to BrainPhys™ with SM1 or maintained in the Neurobasal™ control by performing half-medium changes every 3 - 4 days for 6 weeks. Electrical activities were measured twice a week throughout the culture period. Our data showed that the mean firing rate of neurons in BrainPhys™ cultures increased over time, from 0.03 Hz on day 14, to 1.4 Hz by day 44 (n = 1; 128 electrodes). The percentage of active electrodes (>0.005Hz) also increased from 24% on day 14 to 69% at day 21, and then remained stable at 60 - 70% from days 21 - 44. In contrast, the mean firing rate remained low (<0.1 Hz) in the control, with <5% of electrodes being active over a 6-week period. These data confirm that BrainPhys™ Neuronal Medium with appropriate supplements, supports the survival and synaptic function of hPSC-derived and primary neurons by providing a physiological *in vitro* condition that closely mimics the environment of the brain. BrainPhys™ is therefore, an optimal basal medium for establishing physiologically relevant neuronal cultures for the study of neurobiology *in vitro*.

Disclosures: **C.K.H. Mak:** None. **V.M. Lee:** None. **L.H. Chew:** None. **K. McCormack:** None. **S. Lloyd-Burton:** None. **A.C. Eaves:** None. **T.E. Thomas:** None. **S.A. Louis:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.12/E18

Topic: A.03. Stem Cells and Reprogramming

Support: a grant from the Centre for Biomedical Engineering Research, Toyo University

Title: Effects of environmental changes on iPS cell differentiation and maturation processes

Authors: *Y. YASHIRO, H. MATSUI, H. KAWAGUCHI;
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Abstract: Induced pluripotent stem (iPS) cells have the capacity to differentiate into various cell types, making them useful for clinical applications, such as tissue engineering. In some cases, iPS cells can directly differentiate into functional organs *in vitro*; however, *in vitro* models are generally required to understand the differentiation and maturation processes that occur *in vivo*. In this study, we investigated these processes using a co-culture system of both iPS and primary culture cells derived from mice. In addition, we consider the possibility that both co-culturing and culturing temperatures have a major impact on these processes. Therefore, we investigated the changes occurring in iPS cells when culturing at different temperatures, such as at 39°C or 41°C. First, iPS cells were plated on feeder cells and cultured for 2-3 days. Subsequently, the iPS cells were moved to non-adhesive plastic dishes and cultured for 6 days to form colonies. iPS cell colonies were plated on 24-well assay plates using a cell culture insert and cultured for 6-30 days with dorsal root ganglia (DRG). We measured NeuN protein expression in differentiated neural cells using a fluorescent image analyzer. The NeuN levels in differentiated neural cells, which were derived from co-cultured iPS cells, decreased from 6 to 12 days of culture. Furthermore, βIII-tubulin protein expression in differentiated neural cells decreased from 12 to 18 days of culture. We also analyzed *NeuN* gene expression in differentiated neural cells using real-time PCR. The *NeuN* levels during differentiation in the control transiently peaked at 12 days of culture. In contrast, levels in the co-culture peaked at 6 days of culture. Our results suggest that co-culturing with DRG slightly inhibits iPS cell differentiation into neural cells compared with that in control. However, *NeuN* gene expression levels in the case of co-culture peaked earlier than those in control. Therefore, iPS cell differentiation and maturation processes might be influenced and regulated by other cells, i.e., DRG cells, in the case of co-culture. The protocols used in this study were approved by the Animal Ethics Committee of Toyo University. This work was partially supported by a grant from the Center for Biomedical Engineering Research, Toyo University.

Disclosures: **Y. Yashiro:** None. **H. Matsui:** None. **H. Kawaguchi:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.13/E19

Topic: A.03. Stem Cells and Reprogramming

Title: Development of an online toolkit for xeno-free stem cell research

Authors: *F.-Y. LI[1], A. R. MUOTRI[2], E. L. OHAYON[3], A. LAM[1,3];
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Abstract: Induced pluripotent stem cells (iPSCs) have immense therapeutic potential to treat many dysfunctions in the nervous system. However, iPSCs generation and differentiation remain contaminated with the use of animal-derived products which limits the predictive power stem cell-derived models of human diseases in basic research and restricts their clinical applicability. Significant efforts are now being invested in finding replacements for animal-derived products for iPSC generation and differentiation. To further facilitate the application of these xeno-free techniques, we are creating an open-access online xeno-free stem cell toolkit. This toolkit will include a database of existing xeno-free protocols as well as help identify procedures that require the development of alternatives. The prototype database is being designed to feature advanced search functions and opportunities for users to report reproducibility, optimizations, sample results, ratings and comparisons relative to similar protocols. This interactive online database of xeno-free stem cell protocols is the first resource of its kind and aims to improve accuracy, transparency, quality, cost and rate of translation of stem cell research into clinical applications. Moreover, this framework can be adopted as a protocol centralization model to promote open xeno-free, human-based scientific research with standardized and reproducible methodologies.

Disclosures: **F. Li:** None. **A.R. Muotri:** None. **E.L. Ohayon:** None. **A. Lam:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

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Program#/Poster#: 495.14/E20

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant NS048271

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Title: Forebrain organoids generated using mini-bioreactors for modeling Zika virus exposure and microcephaly

Authors: *X. QIAN[1], H. N. NGUYEN[1], M. M. SONG[1], S. C. OGDEN[2], C. HAMMACK[2], B. YAO[3], J. PENG[3], H. TANG[2], H. SONG[1], G.-L. MING[1];
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Abstract: Differentiation of human induced pluripotent stem cells (iPSCs) into three dimensional (3D) tissue resembling organogenesis, termed organoids, provides a valuable platform for investigating human development *in vitro*. An immediate application of organoid technology is to address the current global public health emergency concerning the link between Zika virus (ZIKV) and microcephaly, a neurodevelopmental disorder, by modeling human brain development. However, high cost, variability and tissue heterogeneity currently limit the accessibility and broad applications of existing brain organoid technologies. To address these challenges, we engineered a miniaturized multi-well spinning bioreactor (SpinΩ) using 3D printing technology, which dramatically reduced the cost of maintaining organoids and permits comparisons of many culture conditions in parallel for optimization. Using two rounds of patterning factor treatments, we developed a protocol to generate forebrain organoids with minimized heterogeneity that enables quantitative analyses and better recapitulation of the

developing human cortex. Importantly, these forebrain organoids exhibit the neural progenitor cell (NPC) organization unique to primates in the form of a well-developed outer subventricular zone-like region containing prominent NPC populations that share molecular and morphological features of human outer radial glia cells (oRGCs). Forebrain organoids also generate robust and organized populations of neurons expressing markers found in all six layers of human cortex, as well as various GABAergic neuronal subtypes. In addition, large-scale comparisons of global transcriptome analyses confirmed that forebrain organoid development closely correlates with human cortical development at the molecular level. Finally, we employed the forebrain organoid platform to model Zika virus (ZIKV) exposure. Quantitative analyses revealed that both African and Asian strains of ZIKV exhibit specific tropism toward NPCs, including oRGCs, although ZIKV could also be detected in immature neurons, intermediate progenitor cells, and astrocytes. Time-course analysis further shows that ZIKV infection in NPCs is productive, resulting in more infected cells over time. ZIKV infection leads to increased cell death and reduced proliferation, resulting in decreased neuronal cell layer volume and overall organoid size, resembling microcephaly. Together, our forebrain organoids and SpinΩ provide an accessible and versatile platform, for modeling human brain development and disease and for compound testing, including potential ZIKV antiviral drugs.

Disclosures: **X. Qian:** None. **H.N. Nguyen:** None. **M.M. Song:** None. **S.C. Ogden:** None. **C. Hammack:** None. **B. Yao:** None. **J. Peng:** None. **H. Tang:** None. **H. Song:** None. **G. Ming:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.15/E21

Topic: A.03. Stem Cells and Reprogramming

Support: FAPERJ

CAPES

CNPq

BNDES

FINEP

FAPESP

Title: Human Radial Glia-like cells are targeted and killed by Zika Virus *In vitro*

Authors: *P. TRINDADE[1], E. C. LOIOLA[2], L. M. HIGA[3], R. MADEIRO DA COSTA[2], J. M. NASCIMENTO[2,4], A. TANURI[3], P. P. GARCEZ[3,2], S. K. REHEN[2,3];
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Abstract: The recent outbreak of Zika virus (ZikV)-associated microcephaly in Brazil is a major concern in terms of public health around the globe. Few data are available regarding the effects of ZikV on the developing brain. Radial glial cells are major neural progenitors during initial stages of central nervous system (CNS) development. Recently, these cells were pointed as potential candidates to mediate ZikV entry in the CNS during development (Nowakowski *et al*., 2016). Here we test the hypothesis that ZikV is able to infect and induce deleterious effects on human radial glia (RG)-like cells derived from induced pluripotent (iPS) stem cells. RG-like cells were obtained by two subsequent differentiation protocols where iPS cells were differentiated into neural stem cells, which then were differentiated as glial cells (Yan et al., 2013). Immunocytochemistry for radial glia markers showed that 99% of these cells are GFAP+/Nestin+ and 90% PAX6+. To test ZikV infectivity, RG-like cells were exposed to two different strains of ZikV (766 and Br_ABES - isolated from African and Brazilian patients, respectively). Immunocytochemistry using the antibody anti-flavivirus group antigen (clone 4G2) was performed after 24h and 48h following virus exposure. In both groups we found that 10% and 30% of cells were labeled with the ZikV antibody, respectively, suggesting that the virus is not only able to infect but also to replicate in these progenitor cells. Cell viability was assessed after ZikV infection (MOIs: 0.25; 0.025; 0.0025). Live/Dead assay (Thermo) was performed using a high-content screening plataform. Three days after infection, significant increase in cell death was observed in the group infected with the 766 ZikV strain (~60% cells died with the 0.25 MOI). At this time point, no significant increase in cell death was observed in the Br_ABES ZikV strain. Six days after infection, we found significant increase in cell death in all different groups infected with the 766 ZikV strain where the 0.25 MOI exhibited ~100% cell death and the groups with 0.025 and 0.0025 exhibited ~70% and 50% dead cells respectively. In this time point we were able to detect that ~60% of cells died in the group infected with 0.25 MOI Br_ABES ZikV strain. Our results point that the 766 ZikV strain is faster than the Br_ABES strain on promoting cell death. The understanding of differences between these two strains of ZikV is crucial to understand the recent outbreak of ZikV-associated microcephaly in Brazil. Taken together these results demonstrate that the model of ZikV infection in RG-like cells is a potential tool for drug screening against the deleterious effects of ZikV in the developing CNS.

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Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.16/E22

Topic: A.03. Stem Cells and Reprogramming

Support: FAPERJ

CAPES

CNPq

BNDES

Finep

FAPESP

Title: Alteration of shape and cell number in human neurosphere infected with pika virus isolated in Brazil

Authors: *R. COSTA[1,2], P. GARCEZ[3,1], J. M. NASCIMENTO[5,1], P. TRINDADE[1], E. LOIOLA[1], L. M. HIGA[4], P. SEQUEIRA[6], G. VITÓRIA[1], J. SOCHACKI[1], A. PHILLIPS[6], A. TANURI[4], S. K. REHEN[1,3];
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Abstract: Zika virus (ZIKV) has been associated with microcephaly, a rare brain malformation characterized by the reduction of cephalic perimeter; Here we examined morphological changes in neurospheres derived from human neural stem cells infected with ZIKV isolated in Brazil. Reduced size, changes in shape and increased cell death mediated by caspase were observed in neurospheres three days after infection. Reduced number neural progenitor cells and neurons were also observed in ZIKV-infected neurospheres. These results suggest human neurospheres is useful to screen for compounds able to reduce the harmful effects of ZIKV infection for neural progenitor cells and developing neurons.

Disclosures: **R. Costa:** None. **P. Garcez:** None. **J.M. Nascimento:** None. **P. Trindade:** None. **E. Loiola:** None. **L. M. Higa:** None. **P. Sequeira:** None. **G. Vitória:** None. **J. Sochacki:** None. **A. Phillips:** None. **A. Tanuri:** None. **S.K. Rehen:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: A.03. Stem Cells and Reprogramming

Support: NINDS 1U24NS095914

NIH 1R01AA023797

Title: Optimization of CRISPR editing for high throughput cell line generation

Authors: J. C. MOORE[1,4,5], M. SWERDEL[2], A. HALIKERE[6], Z. P. PANG[6], *M. SHELDON[3,4,1,5], J. A. TISCHFIELD[1,5,4], R. P. HART[2,4];
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Abstract: The advent of induced pluripotent stem cells (iPSC) has given rise to new tools for modeling human development and disease progression, and for use in drug screening. In one example, the single nucleotide polymorphism (SNP) rs1799971 (OPRM1 A118G) has been linked to drug and alcohol use disorders. Our preliminary analysis of human neurons derived from iPSC revealed functional differences (e.g., ligand sensitivity as well as receptor recycling) between A118- and G118-carrying human neurons from multiple donors. However, the diverse genetic background of the human genome has hampered the usefulness of iPSC in these applications. Currently, control iPSC lines often consist of age and sex matched non-affected subjects or non-affected family members but these exhibit a great deal of heterogeneity due to individual differences. The use of the CRISPR (clustered regularly-interspaced short palindromic repeats)/Cas system can create isogenic cell lines that will serve as better controls. We hypothesize that conversion of the G118 allele to A118 will reverse the phenotypes. We are in the process of implementing CRISPR technology to generate OPRM1 isogenic iPSCs, to further validate this phenotype as a potential biological basis underlying drug abuse behaviors, and as a proof of principle for establishing this technology. Unfortunately, the use of CRISPR/Cas in iPSC is complicated by many factors, including the resistance of iPSC to common exogenous gene delivery methods, the differences between non homologous end joining and homologous recombination in pluripotent vs. somatic cells and the difficulty of obtaining a clonal iPSC population. At RUCDR we are developing a high throughput, cost efficient strategy for using CRISPR/Cas to genetically modify iPSC for creating isogenic lines. Our strategy revolves around the optimization of 4 steps - delivery of the CRISPR/Cas 9 components, gRNA selection,

donor selection and the clonal isolation of a completely edited cell line. Preliminary results indicate that the use of electroporated Cas9 protein pre-bound with in vitro transcribed guide RNA substantially increases DNA cleavage at the desired site. Either synthetic oligonucleotides or plasmids incorporating drug selection strategies may be used as homologous recombination donor. We have adapted methods for screening pooled cells or single-cell-derived colonies. By optimizing each of these steps our goal is to establish a validated protocol for applying CRISPR technology to a broad variety of genetic variants.

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Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.18/E24

Topic: A.03. Stem Cells and Reprogramming

Support: CRM fellowship FP00087081

Title: Biochemical properties and functional effects of apolipoprotein E (apoE) isoforms from iPSC-derived human astrocytes

Authors: *J. ZHAO[1], M. DAVIS[1], Y. ATAGI[1], Z. WSZOLEK[2], N. GRAFF-RADFORD[2], S. YOUNKIN[1], T. KANEKIYO[1], G. BU[1];
[1]neuroscience, [2]neurology, Mayo Clin., Jacksonville, FL

Abstract: Human induced pluripotent stem cells (iPSCs) have recently emerged as a versatile model system to study human diseases. The iPSCs derived from individuals carrying specific gene variants or mutations enable the expression of endogenous genes at the physiological levels, allowing disease modeling of relevant phenotypes *in vitro*. The ε4 allele of the apolipoprotein E (*APOE4*) gene is the strongest genetic risk factor for late-onset Alzheimer's disease (AD) compared to the common ε3 allele or the protective ε2 allele. In the central nervous system, apoE is produced primarily by astrocytes and functions in transporting lipids including cholesterol to support neuronal homeostasis and synaptic integrity. Although mouse models have provided valuable tools for studying apoE isoform-dependent functions, recent studies have shown that human astrocytes have distinct gene expression profile compare with rodent astrocytes. Thus, it is critical to examine isoform-dependent biochemical and functional properties of apoE derived from human astrocytes. We obtained human skin fibroblasts from cognitively normal individuals

with different *APOE* genotypes (ε2/ε2; ε3/ε3; or ε4/ε4) and reprogramed them to iPSC clones by episodic expression of defined transcription factors. The iPSC clones were then differentiated into neural progenitor cells and further into astrocytes. We found that apoE expression appeared in parallel with the appearance of astrocytic markers. Importantly, apoE lipoprotein particles isolated from the conditioned media of human iPSC-derived astrocytes displayed isoform-dependent biochemical properties and functional effects on neuronal viability and synapses. We further converted the *APOE* ε4/ε4 genotype to ε3/ε3 in iPSCs using the CRISPR/Cas9 technology and explored the phenotypic effects on apoE properties and functions. Our studies support apoE isoform-dependent effects using human iPSC-derived astrocytes and provide an in vitro human model in understanding the role of apoE in AD pathogenesis.

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Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.19/E25

Topic: A.03. Stem Cells and Reprogramming

Title: Improved differentiation of human pluripotent stem cell-derived neurons through reduction of progenitor proliferation

Authors: *Y. YAN, N. KAUR, J. SAGAL, P. RAVISHANKAR, M. VEMURI, M. POWERS, D. KUNINGER;
Div. of Cell Biol., Thermo Fisher Scientific, Frederick, MD

Abstract: Neurons derived from human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), are excellent resources for disease modeling and drug screening. Human PSCs derived neural stem cells (NSCs) can be expanded in culture and further differentiated into mature neurons for various experiments. However, cells differentiated from hPSC-derived NSCs often contain mixed population with both differentiated neurons and undifferentiated NSCs by using classical culture medium, typically including a basal medium, B27/N2, brain-derived neurotrophic factor, glial cell-derived neurotrophic factor and other reagents. Due to the continuing proliferation of undifferentiated NSCs, very high cell densities and cell aggregation clumps are usually observed during the differentiation of hPSC-derived NSCs and increase over time, which poses challenges for long-term maintenance and end-point quantification. We have developed supplement which can reduce the proliferation of

undifferentiated NSCs without impacting the rate or extent of differentiation for hPSC-derived NSCs. The overall effect increases the relative population of differentiated neurons in culture. Typically, under these conditions by 2-3 weeks differentiated neurons with extensive neurite networks are seen that are evenly distributed across the culture surface, with very little clumping or aggregation observed. Immunocytochemical staining showed that differentiated neurons expressed mature neuronal marker MAP2 or HuC&D without the contamination of undifferentiated Nestin positive NSCs. Multielectrode array (MEA) showed that differentiated neurons fired spontaneous action potentials, indicating functional neurons. By using this new supplement, differentiated neurons can be maintained for longer time in culture. Furthermore, the evenly distributed mature neurons are more favorable to manual or automated imaging for quantification.

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Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.20/E26

Topic: A.03. Stem Cells and Reprogramming

Title: Activin signaling increases photoreceptor precursor yield during early embryonic stem cell differentiation

Authors: *A. LU[1], C. BARNSTABLE[2];
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Abstract: Activins are members of the transforming growth factor beta family and are involved in stem cell maintenance and differentiation as well as development of multiple organ systems. Specifically, Activin A is expressed during eye field specification and retina development. However, the effect of Activin A on these processes is poorly understood. This is partly due to the difficulty in dissecting and studying the embryonic anterior neural tissue and retina where these early developmental events occur in vivo. In vitro differentiation of pluripotent stem cells provides an alternative approach for studying these developmental events.
We have directed the differentiation of BK3 and HM1 mouse embryonic stem cells (ESCs) into a retinal lineage. The differentiated cultures progress through a series of stages that correspond to the normal progression of development in vivo. These stages include formation of the early eye field, then early retina, and finally generation of specific retina cell types and were identified by

measuring the expression of marker genes. This defined in vitro protocol can be used to investigate the molecular basis for rod photoreceptor development in a strictly controlled in vitro system.
We then used the in vitro differentiation system to test the hypothesis that Activin A increases rod photoreceptor yield during a transient developmental period. Treating BK3 and HM1 ESCs with Activin A results in increased expression anterior neural and eye field marker genes. It also increases and maintains the expression of marker genes associated with photoreceptor precursors and other late retina cell types and decreases the expression of genes associated with early retina cell types and retinal progenitor cells in a dose-dependent manner. This effect is only detectable when Activin A is introduced at stages corresponding to early eye field and retina formation. As these time frames can shift depending on the pluripotent stem cell line used, our results highlight the importance of understanding the variations between pluripotent cell lines and their endogenous levels of Activin expression. Furthermore, while Activin A can increase the proportion of photoreceptor precursors in culture, it does not have an obvious effect on expression of mature rod photoreceptor genes, indicating that Activin A plays a role in eye field specification and retinal lineage determination while other factors may influence maturation of cells committed to the photoreceptor lineage.

Disclosures: **A. Lu:** None. **C. Barnstable:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

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Topic: A.03. Stem Cells and Reprogramming

Support: DoD Grant AZ140064

NIH Grant AG048083-01

Title: Single-cell isolation of human ipsc barcodes derived progenies of neuronal and glial cells

Authors: *A. ALMENAR-QUERALT[1], D. MERKURJEV[2], G. WOODRUFF[1], H.-S. KIM[2], J. E. YOUNG[1], M. PINSACH[2], C. ALLEGUE[2], L. K. FONG[1], C. MACKINTOSH[2], Q. MA[2], L. S. GOLDSTEIN[1], I. GARCIA-BASSETS[2];
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Abstract: Neurons and glial cells derived from genome-edited human induced pluripotent stem cells (e-hiPSCs) have become a popular model to study neurological disorders, but we still lack some basic characterization of these cells as models of disease. While studying the transcriptomes of a panel of e-hiPSC-derived neurons carrying identical Alzheimer's disease-associated mutations, we unexpectedly identified a large number of clonal, genotype-independent disparities among neuronal populations derived from single e-hiPSCs. These disparities originate in the obligatory step of single-cell isolation and selection during the process of genome editing. We observed that the intrinsic cell-to-cell heterogeneity within the parental hiPSC line is transformed into heterogeneity among single cell-derived sublines, and each single cell-derived subline becomes a relatively homogenous subpopulation. This effect is particularly evident in the family of fifty-three human clustered protocadherins (c-pcdhs). Most c-pcdhs are expressed in neurons as single cell-specific combinations of different c-pcdhs. We found that each single cell-derived e-hiPSC subline primes the transcriptional fate of a unique c-pcdh signature (or combination), which is robustly propagated and clonally transmitted into derived neuronal progenitor cells (NPCs), neurons, and glial cells. Single cell-derived, non-edited hiPSCs and blastocyst-derived human embryonic stem cell (hESCs) share this c-pcdh priming property with e-hiPSCs. Since c-pcdh signatures and other clonal attributes inherited from progenitor single hiPSCs may interfere with the identification of genome edited-associated phenotypes when comparing edited and non-edited cells, we will suggest modifications in the genome-editing protocol to circumvent this effect. Corollary to our studies, we concluded that neuronal and glial c-pcdh signatures are, unexpectedly, pre-defined and permanently propagated from an early stage in human pluripotent cells, *barcoding* the progeny derived from the same single cell. These barcodes can be erased and *de novo* written upon cell reprogramming, but remain *locked* afterwards. Intriguingly, neurons derived *in vitro* in the absence of an intermediate pluripotency state (iNs) inherit pre-defined c-pcdh patterns from parental somatic cells, which resemble their non-neuronal, non-pluripotent origin. Since c-pcdh signatures dictate cell-to-cell recognition and self-avoidance during the formation of neuronal circuits, and contribute to neuron diversity in the human brain, our findings add an unexpected and unexplored level of complexity to modeling neurological disease in a dish

Disclosures: **A. Almenar-Queralt:** None. **D. Merkurjev:** None. **G. Woodruff:** None. **H. Kim:** None. **J.E. Young:** None. **M. Pinsach:** None. **C. Allegue:** None. **L.K. Fong:** None. **C. Mackintosh:** None. **Q. Ma:** None. **L.S. Goldstein:** None. **I. Garcia-Bassets:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.22/E28

Topic: A.03. Stem Cells and Reprogramming

Support: CIRM IR1-06600

Title: Induced Pluripotent Stem Cells from the CIRM hPSC Bank collection at the Coriell Institute: A resource for therapy discovery and modeling of neurodevelopmental and neurodegenerative disorders.

Authors: D. HUBER[1,2], C. ZUARES[1], J. HAND[1,2], M. TENORIO[1,2], D. ALTAMURO[1], M. BELLAFANTE[1], L. COSENTINO[1], *C. A. PEREZ[1,2];
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Abstract: Induced pluripotent stem cells (iPSCs) reprogrammed from patient-derived blood or skin cells have become a key resource for human disease modeling and therapy discovery. The California Institute for Regenerative Medicine (CIRM) recognized the value and need for these resources and created the CIRM Human Induced Pluripotent Stem Cell Bank. To ensure the proper storage, handling, and distribution of these samples, CIRM partnered with the Coriell Institute for Medical Research, a leader in biobanking, to fund the establishment of a satellite repository location in California. Coriell provides clinical data management, data hosting, and biospecimen storage and distribution. In addition to several control donors, a variety of genetically complex brain- and eye-related diseases are represented in the CIRM hiPSC Bank: Alzheimer's disease, Macular Degeneration, Glaucoma, Diabetic Retinopathy, Autism Spectrum Disorder, Cerebral Palsy, and Epilepsy. These iPSCs are reprogrammed by Cellular Dynamics International (CDI), which is funded by CIRM to derive iPSC lines from 3,000 donors using their proprietary non-integrating episomal vector system. To ensure the quality of the lines, each one undergoes a rigorous quality control (Chromosomal Integrity, Pluripotency, Identity Confirmation, Loss of Plasmid, Mycoplasma, and Sterility) prior to being banked by the Coriell Institute. A majority of donors are also screened and negative for HIV, HBV, and HCV. The CIRM hPSC Bank is currently comprised of several hundred iPSC lines with extensive clinical data. The specimens are presented to users via Coriell's online (catalog.coriell.org/CIRM) where users have the ability to perform detailed clinical data searches, browse dedicated disease and sample pages and place orders. Importantly, each of these iPSCs can be fully licensed to afford commercial entities Freedom to Operate. The overall quality of these lines, Freedom to Operate, available clinical data, and accessible catalog experience makes this an unmatched resource for researchers worldwide. The CIRM hPSC Bank at Coriell contributes to fulfill CIRM's mission of

accelerate stem cell treatments to patients with unmet medical needs by making high quality iPSCs publicly available worldwide to investigators from academia and industry.

Disclosures: D. Huber: None. **C. Zuares:** None. **J. Hand:** None. **M. Tenorio:** None. **D. Altamuro:** None. **M. Bellafante:** None. **L. Cosentino:** None. **C.A. Perez:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

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Topic: A.03. Stem Cells and Reprogramming

Support: NIH Award 5R24HD000836

NIH Award 5DP1MH099906-03

NSF Grant PHY-0952766

Title: Mapping human brain development and lineage using embryonic stem cells

Authors: *B. P. LEVI[1], C. L. THOMPSON[1], S. KU[1], V. MENON[1], J. K. MICH[1], Z. YAO[1], L. FURCHTGOTT[2], A.-R. KROSTAG[1], R. A. MARTINEZ[1], H. MULHOLLAND[1], S. BORT[1], B. W. GREGOR[1], R. D. HODGE[1], A. M. NELSON[1], N. K. NGO[1], N. S. SHAPOVALOVA[1], E. R. THOMSEN[1], I. A. GLASS[3], A. KAYKAS[1], J. W. PHILLIPS[1], J. S. GRIMLEY[1], Y. WANG[1], S. RAMANATHAN[2];
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Abstract: The human brain is a profoundly complex organ composed of billions of neurons, representing tens to hundreds of interconnected cell types, and elegantly assembled into an organ capable of consciousness, reason, and personality. Advances in pluripotent stem cell technologies, and their use in the creation of human cortical neurons provides a promising platform to study human brain development and associated neurodevelopmental disorders. Here, we present an analysis of the lineage of human cortical cell types generated from differentiated human embryonic stem cells (hESCs), and a comparison of those cell types with primary samples. Single progenitors and neurons were transcriptionally profiled throughout the differentiation time-course. Comparison of the transcriptomic data to existing gene expression atlases and primary human fetal tissue putatively identified a major lineage branch in the culture as belonging to the earliest born neurons of the cortex deriving from the preplate, including

Cajal-Retzius and/or subplate neurons. New computational methods were developed to infer a lineage tree from the single-cell gene expression data, highlighting a major early branchpoint dividing the lineage of cortical, preplate-like neurons from a different branch exhibiting a set of mid/hindbrain gene markers, suggesting that the earlies molecular steps in establishing region-specific neural lineages are represented in this culture. In summary, through comprehensive single-cell transcriptomic profiling, we present a hESC-derived lineage tree of multiple brain regions and demonstrate its similarity to progenitors and neurons found in primary tissues.

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Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.24/E30

Topic: A.03. Stem Cells and Reprogramming

Support: National Natural Science Foundation of China Grants (81471301)

Title: Modeling GABAergic interneuron deficits in Down syndrome using patient iPSCs

Authors: ***Y. LIU**[1], Z.-Y. QU[1], H.-Q. HUO[1], A. BHATTACHARYYA[2], L. MA[3], F. YUAN[1], M. XU[1], Y. HU[1], S.-C. ZHANG[2];
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Abstract: Down syndrome (DS) is the most common genetic disorder of intellectual impairment and is caused by trisomy of chromosome 21. Although DS patient exhibit reduced brain size and diminished GABA transmitter from fetal tissue and postmortem studies, the precise cellular and developmental mechanisms of cognitive disorder in DS remain unclear. Patient induced pluripotent stem cells (iPSCs) provide an opportunity to uncover human developmental disorders. Here, we differentiated iPSCs derived from DS patients into forebrain GABA interneurons. Compared to the disomy iPSC control, the DS GABA interneurons demonstrated

defects of neuronal morphological complexity in vitro. To discern the cellular behaviors of DS GABA interneurons in vivo, we further transplanted these GABAergic progenitors into medial septum of SCID mice. After six months of transplantation, the transplanted DS iPSCs generated GABA interneurons similarly with disomy iPSCs in vivo. However, we found DS GABA interneurons had a reduction of neural soma size, shorter neurites, and less dendritic complexity in the graft and adjacent area. Furthermore, DS GABA interneurons exhibit impaired migration and neurite projection along septohippocampal pathway and in hippocampus. These results suggest the abnormal development of DS GABA interneurons may connect to the diminished GABA transmitter and reduced brain size in DS patients. Our study illustrated pathological features of DS and offered a novel model to study the mechanisms of Down syndrome.

Disclosures: **Y. Liu:** A. Employment/Salary (full or part-time): Nanjing Medical University, University of Wisconsin, Madison. **Z. Qu:** None. **H. Huo:** None. **A. Bhattacharyya:** None. **L. Ma:** None. **F. Yuan:** None. **M. Xu:** None. **Y. Hu:** None. **S. Zhang:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.25/E31

Topic: A.03. Stem Cells and Reprogramming

Title: Zika virus impairs growth and alters cell cycle and neurogenic programmes

Authors: ***P. P. GARCEZ**[1], J. MOTA DE VASCONCELOS[3], R. MADEIRO DA COSTA[4], E. C. LOIOLA[4], R. DELVECCHIO[2], P. TRINDADE[4], L. H. HIGA[2], J. M. NASCIMENTO[5], J. J. VIANEZ[3], A. TANURI[2], S. K. REHEN[4];
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Abstract: INTRODUCTION:
Zika virus (ZIKV) has been associated with microcephaly and other brain malformations; however, the molecular effects of ZIKV over the central nervous system remain to be uncovered. Here we examined the deregulated genes expressed in neurospheres infected with ZIKV.
METHODS & RESULTS:
Human Neral Stem Cells (NSC) were exposed to ZIKV Br_AB_ES- isolated from Brazilian patients. After 2 hours viral exposure (0.025 MOI), NSCs were submitted to a differentiation protocol to form neurospheres. Immunocytochemistry was performed 3 days after ZIKV

exposure. To confirm ZIKV infection, we used flavivirus group antigen (clone 4G2) antibody. Apoptosis was measured with activated caspase immunofluorescence. Three days after infection, there was a significant increase in cell death observed in ZIKV infected neurosheres. After 12 days in vitro, neurospheres were completely depleted. In order to evaluate molecular effects of ZIKV infection, we performed RNA-seq of the infected and mock neurospheres after 3 days in vitro. mRNA transcriptional profile analyses revealed that ZIKV infection alters cell cycle regulators and downregulates neurogenic programmes, in addition to transcriptional regulation due to viral replication.

CONCLUSIONS:

Altogether, ZIKV infection modulated genes involved in different pathways inhibiting cell cycle and neurogenesis. The effect of ZIKV at crucial steps of brain development likely explains the consequences of the so-called congenital syndrome of ZIKV on brain formation and function. Our work provides insights into the molecular mechanisms of ZIKV infection. It implicates the ZIKV in progenitor proliferation, differentiation and survival, thus improving our understanding of human microcephalies.

Disclosures: **P.P. Garcez:** A. Employment/Salary (full or part-time): Federal University of Rio de Janeiro. **J. Mota de Vasconcelos:** A. Employment/Salary (full or part-time): Evandro Chagas Institute. **R. Madeiro da Costa:** A. Employment/Salary (full or part-time): D'Or Institute for Research and Education (IDOR). **E.C. Loiola:** A. Employment/Salary (full or part-time): D'Or Institute for Research and Education (IDOR). **R. Delvecchio:** None. **P. Trindade:** A. Employment/Salary (full or part-time): D'Or Institute for Research and Education (IDOR). **L.H. Higa:** None. **J.M. Nascimento:** None. **J.J. Vianez:** A. Employment/Salary (full or part-time): Evandro Chagas Institute. **A. Tanuri:** A. Employment/Salary (full or part-time): Federal University of Rio de Janeiro. **S.K. Rehen:** A. Employment/Salary (full or part-time): Federal University of Rio de Janeiro.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.26/E32

Topic: A.03. Stem Cells and Reprogramming

Support: Fondation Toulouse Cancer Sante

Title: From neuronal progenitors to human neuronal disease models: a 3D path.

Authors: J. CHOUINARD[1], K. KOSIK[2], *S. PAUTOT[1];
[1]ITAV- CNRS USR3505, Toulouse, France; [2]Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: To help delineate pathways for CNS diseases few challenges in growing and maintaining in vitro neuronal network from human neuronal progenitor cells need to be resolved. The main hurtles come from the difficulties to grow, maintain and differentiate human progenitors cells. To contribute to a standardization of neuronal network recording, we show that, in a 3D environment, neuronal progenitors can autonomously differentiate in all three neuronal cell types to replicate in vivo cell architecture, cell composition, and functional activity. We have first established that neuronal networks obtained with rat neuronal progenitors displayed earlier spontaneous neuronal activity and long-term viability compared to standard in vitro models. We then demonstrated that these findings extended to human neuronal progenitors cells. Our experiments showed that human neuronal progenitors derived from hIPSCs could grow and differentiate in our 3D culture system to display spontaneous neuronal activity after four weeks in culture. Using the expression of fluorescent proteins under neuronal cell specific promoters (nestin and synapsin) we monitored the cell differentiation process and the network formation. In parallel, we recorded the evolution of the spontaneous spiking activity over 8 weeks and follow the developmental stage of the neuronal activity. These networks exhibited a long-term viability with no apparent decay in activity for up to 8 weeks. Furthermore, localized electrical stimulation allowed us to establish the existence of long-range connections with specific response patterns depending on both the location of the stimulation and its frequency as expected in in vivo networks. Our results suggest that this approach could contribute to a standardization of human neuronal network formation and become instrumental in the study human neurological disorder by providing a mean to monitor simultaneously neuronal differentiation and neuronal network activity for patient derived iPSCs.

Disclosures: J. Chouinard: None. **K. Kosik:** None. **S. Pautot:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.27/E33

Topic: A.03. Stem Cells and Reprogramming

Support: FAPESP 2014/21035-0

Title: Unraveling the proteomics of ZIKV-infected neurospheres

Authors: *J. M. NASCIMENTO[1,2], P. P. GARCEZ[3,2], J. S. CASSOLI[1], J. M. VASCONCELOS[4], R. MADEIRO DA COSTA[2], P. TRINDADE[2], E. C. LOIOLA[2], L. H. HIGA[3], J. SOCHACKI[2], G. VITORIA[2], A. TANURI[3], D. MARTINS-DE-SOUSA[1], S. K. REHEN[2];
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Abstract: After the recent outbreak of Zika virus (ZikV) in Brazil, this has been associated with microcephaly and other brain malformation, and is a major concern in terms of public health around the globe. Nevertheless, despite increasing efforts to understand the effects of ZIKV infection in the central nervous system, much of the molecular outcomes remain uncovered. Our aim was to analyse the molecular consequences of ZIKV infection in neurospheres derived from human induced pluripotent stem cells. These three-dimensional structures contain diving neural progenitors proliferating undergoing neuronal differentiation, glial cells and neurons. Global protein expression was obtained by state-of-the-art quantitative label-free shotgun proteomics. Comparing the expression of the most differentially regulated proteins ($p<0.05$) in ZIKV-infected neurospheres versus mock neurospheres, we detected 199 downregulated and 259 upregulated proteins, mainly involved with translational and transcriptional machinery and cell cycle. To get further insight into these pathways, we analysed the interactome of those proteins, which indicated G1 arrest of the cell cycle progression and the impairment of the neuronal program. These results suggest that zika virus stops cell cycle progression, depleting the pool neural progenitor cells during brain development.

Disclosures: J.M. Nascimento: None. **P.P. Garcez:** None. **J.S. Cassoli:** None. **J.M. Vasconcelos:** None. **R. Madeiro da Costa:** None. **P. Trindade:** None. **E.C. Loiola:** None. **L.H. Higa:** None. **J. Sochacki:** None. **G. Vitoria:** None. **A. Tanuri:** None. **D. Martins-de-Sousa:** None. **S.K. Rehen:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.28/E34

Topic: A.03. Stem Cells and Reprogramming

Support: CNPq - Brazil

FAPERJ

CAPES

BNDES

FINEP

FAPESP

Title: Zika virus affects cell viability of human neural stem cells

Authors: *E. C. LOIOLA[1], P. TRINDADE[1], L. M. HIGA[2], R. MADEIRO DA COSTA[1], J. M. NASCIMENTO[1,4], A. TANURI[2], P. P. GARCEZ[1,3], S. K. REHEN[1,3];
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Abstract: Reports of microcephaly in Brazil in combination with Zika outbreak suggests a link between virus infection and brain malformations. Here, we analyzed if the infection with different strains of Zika virus (ZIKV) could reduce human neural stem cells (NSCs) viability as a potential candidate to mediate deleterious effects of this virus on developing brain. We also investigated the cytotoxic potential of Dengue virus, another flavivirus that circulates in Brazil, on NSCs.Human induced pluripotent stem cells were differentiated as NSCs. To test virus infectivity, NSCs were exposed to two different strains of ZIKV (766 and Br_PE - isolated from African and Brazilian patients, respectively, at 0.25 MOI) and also Dengue Virus (DENV2). Immunocytochemistry using the antibody anti-flavivirus group antigen (clone 4G2) was performed after 24h and 72h following virus exposure. In ZIKV 766 strain and DENV2 we observed 21% and 27 % of cells labeled with anti-flavivirus antibody after 24h, although ZIKV Br_PE could only be detected after a longer time post-infection, and 30% of NSCs were labeled after 72h. Cell viability was assessed after ZIKV infection with a 0.025 MOI. Live/Dead assay (Thermo) was performed in a high-content screening plataform. Three days after infection, significant increase in cell death was observed in the group infected with 766, but not with DENV2. Dead cells corresponded to 4% in Mock controls and 58% and 8% in 766 and DENV2, respectively. Dying cells labeled with an active caspase antibody correspond to 7, 14 and 8% in Mock, 766 and DENV2 groups, respectively.Our results point that the 766 ZIKV strain is able to promote NSCs death, whereas DENV2, another endemic flavivirus in Brazil did not induce significant cell death or caspase activation on similar time points. The Brazilian strain Br_PE of ZIKV showed a slower infection rate, suggesting that there are differences between these two strains of ZIKV that could be related with the increased microcephaly cases in Brazil. Taken together these results demonstrate that ZIKV infection induces cell death in NSCs, which can account to the deleterious effects of ZIKV in the developing CNS.

Disclosures: **E.C. Loiola:** None. **P. Trindade:** None. **L.M. Higa:** None. **R. Madeiro da Costa:** None. **J.M. Nascimento:** None. **A. Tanuri:** None. **P.P. Garcez:** None. **S.K. Rehen:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.29/E35

Topic: A.03. Stem Cells and Reprogramming

Support: CA61393

Title: Use of stem cell derived neurons to study chemotherapeutic induced neuropathy

Authors: *M. DOLAN[1,2], S. DELANEY[2], C. WING[2];
[1]Med., [2]Univ. of Chicago, Chicago, IL

Abstract: Chemotherapeutic-induced peripheral neuropathy (CIPN) is one of the most common and potentially permanent side effects of chemotherapy, second only in frequency to hematopoietic toxicity. CIPN often times irreversible, is a condition with pain, numbness, tingling and sensitivity to cold/hot in hands and feet. About 20-40% of cancer patients with estimates of about 400,000 patients each year develop CIPN as a consequence of treatment with platinum analogues (cisplatin, oxaliplatin, carboplatin), taxanes (paclitaxel, docetaxel), vinca alkaloids (vincristine), thalidomide, epothilones and bortezomib. Moreover, CIPN can lead to dose reduction of the chemotherapeutic agent or possible cessation of treatment. This may have an adverse impact on cancer treatment and disease outcomes. The severity of CIPN is greater among older patients and African Americans. Clinical genome wide association studies of CIPN following treatment with paclitaxel, docetaxel, vincristine and cisplatin have been conducted in efforts to identify genetic variants associated with CIPN; however there is a need for appropriate model systems to determine which associations are the true signals, what mechanisms are responsible, and if they represent treatment opportunities. We have employed the use of induced pluripotent stem cell derived neurons to functionally validate genetic variants/genes identified in genome wide studies of CIPN. We have optimized conditions to identify reproducible differences in relative neurite outgrowth phenotypes following treatment with neurotoxic chemotherapeutics including, but not limited to thalidomide, paclitaxel, vincristine, cisplatin, cannibidiol (component of marijuana) and bortezomib. Similarly to that observed in patients, neurotoxic doses of vincristine are approximately 40-fold lower than those for paclitaxel and 75-fold lower than those for cisplatin. In addition, prior animal studies reveal similar differences in severity among drugs compared to our results. The iPSC-derived neurons allow: a) studies of peripheral neuropathy in human neurons in ways that were not possible previously; b) mechanistic insight on druggable targets to treat or prevent this devastating side effect of chemotherapy; c) provide a model to study genetic components and genes contributing to severe neurotoxicity; d) provide a genetically diverse model that can be used to develop cell based

phenotypic assays for high content screening of all new drugs to determine if they are neurotoxic.

Disclosures: **M. Dolan:** None. **S. Delaney:** None. **C. Wing:** None.

Poster

495. In Vitro Models of Human Brain Development and Pathology: From Stem Cells to Brain Organoids

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 495.30/E36

Topic: A.03. Stem Cells and Reprogramming

Title: Inducing human embryonic stem cells to differentiate into neuroectoderm by suppressing a novel gene expression

Authors: ***J.-J. LIU**[1], S.-C. YANG[2], J. LU[3];
[1]Univ. of Pennsylvania, Sch. of Med., Philadelphia, PA; [2]Inst. of Biochem. and Mol. Biol., Natl. Yang-Ming Univ., Taipei, Taiwan; [3]Academia Sinica, Taipei, Taiwan

Abstract: Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSC) are characterized by their robust expansion ability and pluripotency. However, the application of ESC- or iPSC- derived cells is hampered by the low efficiency of differentiation. To reveal the genes inhibit ESC differentiation, a shRNA functional screen is performed with 517 shRNA targets 121 genes. Based on the results of ESC undifferentiated marker alkaline phosphatase activity assay and the relative cell number Alamer blue assays, 21 genes altered ESC pluripotency were identified. Among these hits, the downregulation of one candidate gene, ATF1, can promote ectoderm differentiation by the upregulation of early neuroectoderm genes SOX2 and PAX6 within 3 days with nearly 100% efficiency. Conversely, the overexpression of ATF1 inhibits the upregulation of SOX2, which is required for neuron induction. We also found that ATF1 can bind to the promoter regions of SOX2 and PAX6 by CHIP-PCR assay. These results suggested ATF1 may serve as a suppressor by directly binding to Sox2 and Pax6 promoters. We will further reveal the molecular mechanisms of how ATF1 inhibits neuroectoderm differentiation by the regulating SOX2 and PAX6. The downregulation of ATF1 with shRNA or siRNA provided an efficient method to generate neural lineage cells that might be useful for regenerative medicine in the future.

Disclosures: **J. Liu:** None. **S. Yang:** None. **J. Lu:** None.

Poster

496. Rett Syndrome

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 496.01/E37

Topic: A.07. Developmental Disorders

Support: ANR ANTARES

INSERM

Aix-Marseille Université

AFSR

Title: Phosphorylation of huntingtin at ser421 modulates the phenotype of the mecp2 deficient mice

Authors: *Y. EHINGER[1], L. SAIDI[1], V. MATAGNE[1], J. BRUYÈRE[2], F. SAUDOU[2], J.-C. ROUX[1], L. VILLARD[1];
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Abstract: Rett syndrome is a neurological disorder caused by mutations in the X-linked MECP2 gene. We demonstrated that Huntingtin-dependant (Htt) axonal transport is altered when Mecp2 is lacking partly due to a deficit of the molecular motor contents (Roux et al., 2012). However, the neuronal trafficking is also strongly dependent on the phosphorylation level of Htt at serine 421 (S421) (Zala et al, 2008). Therefore, we developed several tools in order to stimulate pharmacologically Htt phosphorylation at S421 in vivo and in vitro using: 1) a direct activation of the Akt pathway, through the stimulation of the insulin/IGF1 receptors and 2) the indirect blocking of the Htt dephosphorylating using Fk506. In a second step we used a genetic approach by crossing Mecp2 deficient mice with knockin mice expressing either an aspartic acid or alanine at position 421 to mimic constitutive phosphorylation (S421D) or to prevent phosphorylation (S421A), respectively. For both pharmacology and genetic crossing we used a battery of behavioral tests : grip strength, rotarod, open field and the respiratory profile from disease onset. Our findings demonstrate that pharmacological and genetic stimulation approaches increase longevity in Mecp2 deficient mice. Altogether, behavioural tests suggest a reduction of motor and respiratory impairments. Further studies are necessary to evaluate the neuronal trafficking at a molecular level. Our results indicate that stimulation of the Htt S421 represents a promising way to improve the neuronal trafficking in RTT and a possible target to develop treatments in RTT.

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Poster

496. Rett Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH Grant R01-NS065027

NIH Grant R21-HD074418

Rettsyndrome.org

Rett Syndrome Research Trust

Title: Stronger contribution and impaired LTP of hippocampal inputs to the medial prefrontal cortex in the Mecp2 mouse model of Rett syndrome

Authors: *M. PHILLIPS, W. LI, L. POZZO-MILLER;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: The balance between excitation and inhibition (E/I) in the CNS is crucial for proper brain function. This balance is altered in several brain regions of mouse models of Rett syndrome, a neurodevelopmental autism spectrum disorder caused by mutations in the methyl-CpG binding protein-2 (*MECP2*) gene. E/I imbalance results in altered levels of neuronal activity, causing neural networks to be either hyper or hypoactive. We quantified network activity by imaging voltage-sensitive dye (VSD) signals in slices from symptomatic male *Mecp2* knockout (KO) mice and wildtype (WT) littermates. Evoked VSD signals are larger, longer lasting, and wider spreading in slices of the ventral hippocampus (vHipp) from *Mecp2* KO mice, compared to WT slices. In contrast, VSD signals in slices from the medial prefrontal cortex (mPFC) of *Mecp2* KO mice are shorter lasting and less spreading. These data are confirmed by the number of neurons expressing the immediate early gene c-Fos: the *Mecp2* KO vHipp has more c-Fos positive neurons, while the *Mecp2* KO mPFC has fewer c-Fos neurons. Intriguingly, stimulation of identified vHipp fibers in mPFC slices evoked larger and wider spreading VSD signals in *Mecp2* KO slices. Normalized to intra-cortical stimulation in the same slices, there is a stronger contribution of vHipp inputs to the *Mecp2* KO mPFC (93% of intra-cortical responses *vs*. 71% in WT). Altogether, these data suggest that vHipp fibers drive hyperactivation of the mPFC network in *Mecp2* KO mice, in contrast to the hypoactivity evoked by stimulation of

intra-cortical fibers. In addition, high-frequency stimulation of vHipp fibers triggers an enduring enhancement of the duration and spread of VSD signals in mPFC slices from WT mice, which is reminiscent of long-term potentiation (LTP) of synaptic potentials. This LTP of VSD signals at vHipp-mPFC synapses is absent in *Mecp2* KO mice, which may contribute to behavioral deficits during their social interaction with known and unknown mice. Current experiments are designed to identify the postsynaptic receptors responsible for VSD signals evoked by either stimulation of vHipp inputs or intra-cortical fibers, and whether LTP of VSD signals at vHipp-mPFC synapses in *Mecp2* KO slices is saturated by already potentiated synapses, as occurs in the hippocampus. Supported by: R01-NS065027, R21-HD074418, Rettsyndrome.org, and Rett Syndrome Research Trust to LP-M.

Disclosures: **M. Phillips:** None. **W. Li:** None. **L. Pozzo-Miller:** None.

Poster

496. Rett Syndrome

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Topic: A.07. Developmental Disorders

Support: Cluster of Excellence and DFG Research Center Nanomicroscopy and Molecular Physiology of the Brain (CNMPB)

Title: Deciphering oxidative stress in rett syndrome with genetically-encoded redox sensors

Authors: K. CAN, K. WAGENER, B. KOLBRINK, K. DIETRICH, K. BELINDA, *M. MUELLER;
Univ. Goettingen, Goettingen, Germany, Germany

Abstract: Rett syndrome (RTT) is a progressive neurodevelopmental disorder which affects almost exclusively girls. After a largely normal development in the first year, a complex clinical phenotype manifests. Characteristic symptoms include cognitive impairment, epilepsy, motor dysfunction, and severe breathing disturbances. There is convincing evidence that mitochondrial function is affected in Rett syndrome and that is contributes to the occurrence of oxidative stress. Genetically-engineered optical sensors such as reduction oxidation sensitive green fluorescent protein (roGFP) enable a quantitative assessment of subcellular redox conditions. Detailed redox analyzes in isolated brain tissue of male Rett mice revealed intensified mitochondrial respiration, less efficient cellular redox homeostasis, and oxidative stress in cytosol and mitochondria of MeCP2-deficient neurons. Interestingly, these alterations occur already at neonatal stages, long before first symptoms arise. Therefore, the redox imbalance may critically contribute to the

manifestation of typical Rett symptoms and facilitate disease progression. So far, the mandatory transfection/transduction mostly restricted redox imaging to cultured preparations. To assess subcellular redox conditions also in adult tissue and complex preparations, we generated transgenic redox indicator mice. They express roGFP under the control of the Thy-1.2 promoter in either cytosol or mitochondrial matrix. The roGFP redox sensor is fully functional and almost abundantly expressed in excitatory projection neurons throughout the brain. Currently, roGFP mice are cross-bred with MeCP2-deficient mice. First offspring has already confirmed that the transgene is forward and that the redox balance is also more oxidized in hippocampal neurons of adult, symptomatic male Rett mice. Availability of these roGFP-Rett mice will now reveal subcellular redox conditions during normal maturation and the different stages of Rett syndrome. The extent of redox alterations can be quantified reliably and correlated in detail with disease progression. This will not only allow to map and to identify those brain regions being most severely affected by the redox imbalance, but also to clarify to what extent redox alterations and the resulting oxidative stress drive the progression of this neurodevelopmental disorder. Furthermore, a valuable system is now available to confirm the feasibility of antioxidant/radical scavenger based therapeutic approaches for the treatment of Rett syndrome.

Disclosures: K. Can: None. **K. Wagener:** None. **B. Kolbrink:** None. **K. Dietrich:** None. **K. Belinda:** None. **M. Mueller:** None.

Poster

496. Rett Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH Grant R01-NS065027

IRSF-3117

Title: EEA1 overexpression reduces synaptic strength and restores homeostatic synaptic plasticity in cultured hippocampal neurons from Mecp2 knockout mice

Authors: *X. XU[1], L. POZZO-MILLER[2];
[1]Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL; [2]Neurobio., The Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Negative-feedback homeostatic synaptic plasticity (HSP), also known as synaptic scaling, maintains the global synaptic strength of individual neurons in response to sustained

alterations in neuronal activity. This cell-wide homeostatic balance is critical to allow the potentiation or depression at small subsets of synapses during positive-feedback synaptic plasticity (i.e. Hebbian plasticity). Rett syndrome (RTT) is a progressive autism spectrum disorder caused by loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2). Previously, we discovered an imbalance of synaptic excitation and inhibition (E/I) in the hippocampus of symptomatic *Mecp2* knockout (KO) mice (Calfa *et al.* 2015). Since E/I balance is thought to be maintained by homeostatic mechanisms, we examined the role of MeCP2 in HSP at the excitatory synapses. We found that hippocampal neurons obtained from P1 *Mecp2* KO mice (9-11 days *in vitro*) do not show the characteristic homeostatic scaling-up of mEPSC amplitude and of synaptic levels of GluA1 after 48hs silencing with the Na^+ channel blocker TTX. This deficit in HSP is bidirectional because *Mecp2* KO neurons also failed to scale-down mEPSC amplitude and synaptic levels of GluA1 after 48hs of desinhibition with the GABA-A receptor antagonist bicuculline. The best-characterized mechanism of HSP is the regulated trafficking into and out of synapses of the AMPA-type of glutamate receptors (AMPARs). We focused on early-endosome-antigen-1 (EEA1) because it participates in synaptic removal of GluA1 (Selak *et al.* 2006), and was found to be activated by MeCP2 in a microarray study (Chahrour *et al*. 2008); consistently, mRNA and protein EEA1 levels are lower in the *Mecp2* KO hippocampus (Li *et al.* 2016). Here, we tested whether EEA1 overexpression restores mEPCS amplitudes and HSP in hippocampal neurons from *Mecp2* KO mice. EEA1 overexpression in *Mecp2* KO neurons reduced mEPSC amplitudes to levels comparable to those in WT neurons. In addition, *Mecp2* KO neurons overexpressing EEA1 scaled-down mEPSC amplitudes after desinhibition with bicuculline, suggesting that HSP is restored. Our characterization of the role of EEA1 during HSP in *Mecp2* KO neurons provides novel targets for improving hippocampal function in RTT individuals.

Disclosures: X. Xu: None. **L. Pozzo-Miller:** None.

Poster

496. Rett Syndrome

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Topic: A.07. Developmental Disorders

Support: Rett Syndrome Research Trust

Scottish Rett Syndrome Association

Chief Scientist Office

Rosetrees Foundation

RS Macdonald Charitable Trust

Title: Improved survival in a knock-in model of Rett syndrome after systemic administration of AAV9/MECP2

Authors: *S. R. COBB[1], K. K. GADALLA[1,2], T. VUDHIRONARIT[1], N. BAHEY[1,3], R. HECTOR[1], M. E. S. BAILEY[1], S. GRAY[4];
[1]Univ. of Glasgow, Glasgow, United Kingdom; [2]Pharmacol. Dept., [3]Histology Dept., Tanta Univ., Tanta, Egypt; [4]Dept. of Ophthalmology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: *De novo* mutations in the X-linked gene *MECP2* are responsible for ~95% of typical Rett syndrome (RTT) cases which are characterized by severe motor and cognitive impairments in females. The missense T158M mutation is one of the most common and severe RTT-causing mutations. It is unknown whether the presence of endogenous mutated protein may impede the therapeutic action of a gene therapy approach. The aim of this study was therefore to investigate the effectiveness of systemic administration of scAAV9/h*MECP2* on survival and other phenotypes in the *Mecp2*$^{T158M/y}$ knock-in mice.
A myc-tagged human *MECP2_e1* minigene construct packaged in a self-complementary AAV2/9 vector (10^{12} vg/mouse) was delivered intravenously into juvenile *Mecp2*$^{T158M/y}$ mice which were then monitored for survival, body weight and RTT-like phenotype severity score. Treated *Mecp2*$^{T158M/y}$ mice had significantly extended survival (p =0.001) and increased body weight (p <0.001) compared to vehicle-treated mice. However, there was no improvement in the aggregate severity score. Brain transduction efficiency was relatively low (1-3% of DAPI-positive cells), which likely explains the lack of impact on neurological phenotypes. Similar results were obtained in *Mecp2*$^{-/y}$ KO mice.
These data support the concept that the action of exogenous MeCP2 is unlikely to be confounded by deleterious interactions with endogenous mutated MeCP2 protein. Currently, we are optimizing the route of administration and vector design to enhance brain transduction and maximize therapeutic efficacy.

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Poster

496. Rett Syndrome

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Topic: A.07. Developmental Disorders

Support: R01JH076901, NICHD, NIH

Title: Novel dendrimer-conjugated glutaminase inhibitor targets glutamate dysregulation in a mouse model of Rett Syndrome

Authors: *E. S. SMITH[1], R. R. REDDY[2], S. P. KAMBHAMPATI[2], F. ZHANG[2], M. V. JOHNSTON[3,4], B. SLUSHER[3,5], R. M. KANNAN[1,2,3], M. E. BLUE[3,4], S. KANNAN[1]; [1]Critical Care Med., [2]Ctr. for Nanomedicine, [3]Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [4]Hugo W. Moser Res. Inst., Kennedy Krieger, Baltimore, MD; [5]Johns Hopkins Drug Discovery, Baltimore, MD

Abstract: Glutamate dysregulation plays a neuropathological role in Rett Syndrome (RTT) and in mouse models of RTT. Previous studies have shown that increased glutaminase levels in microglia from *Mecp2*-null mice lead to overproduction of glutamate and cellular toxicity. This combined with other coinciding neuropathology could potentially contribute to cell stress and toxicity as well as neurobehavioral consequences associated with RTT. Glutaminase inhibition has a potential therapeutic avenue for targeting diseases where glutamate levels are excessive. However, glutaminase inhibitors are poor drug candidates for central nervous system applications since they have poor solubility and do not cross the blood brain barrier (BBB). To investigate the use of dendrimer-mediated therapy, we used dendrimer-conjugated to a novel Bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl Sulfide 3 analog, JHU-29 (D-JHU-29) in a *Mecp2*-null mouse model of RTT. We first injected generation-4 poly (amidoamine) dendrimers tagged with a fluorescent tag (Cy5; D-Cy5) to determine whether dendrimer nanoparticles penetrate the BBB of *Mecp2*-null mice. Twenty-four hours after injection, D-Cy5 was found to be specifically co-localized in microglia and astrocytes of *Mecp2*-null mice. Secondly, we evaluated the efficacy of D-JHU-29 *ex vivo* to reduce extracellular glutamate (Glu) levels in hippocampal slice culture from *Mecp2*-null mice. The results showed increased Glu levels in cultures from *Mecp2*-null mice compared to those from wild type mice and that D-JHU-29 treatment lowered Glu levels. Further studies are ongoing that will determine the impact of D-JHU-29 in *Mecp2*-null mixed glial cultures and the acute *in vivo* efficacy of D-JHU-29 in reducing phenotypic features of *Mecp2*-null mice. Our preliminary results indicate that dendrimer-mediated glutaminase inhibition may be a viable treatment approach for reducing glutamate-related neuropathology in RTT.

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Poster

496. Rett Syndrome

Location: Halls B-H

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Topic: A.07. Developmental Disorders

Support: International Rett Syndrome Foundation

NINDS 1F32NS083137-01A1

NINDS 5R01NS057819

Title: Pharmacological modulation of GABAergic neurons in a mouse model of Rett Syndrome

Authors: *K. M. URE, H. ZOGHBI;
Baylor Col. of Med., Houston, TX

Abstract: Rett syndrome is a postnatal neurological disorder caused by mutations in the gene encoding methyl-CpG binding protein 2 (*MECP2*) and is characterized by ataxia, learning and memory deficits, seizures, breathing abnormalities, and a stereotyped hand-wringing motion. Mice with *Mecp2* deleted from the brain replicate most of the human symptoms. Surprisingly, loss of *Mecp2* specifically from GABAergic neurons replicates most of these phenotypes, and reexpression of *Mecp2* solely in GABAergic neurons reverses many of the disease phenotypes. These findings highlight the importance of proper inhibitory neuronal function in Rett syndrome and suggest that pharmacological enhancement of inhibition may be a therapeutic approach for the disorder. Here, we report the results of two drug trials in *Mecp2*-null male and *Mecp2*-heterozygous female mice: vigabatrin, which irreversibly blocks GABA catalysis, thus increasing somatic GABA content, and SGE-516, a novel neurosteroid that acts as a positive allosteric modulator of the $GABA_A$ receptor. We injected male wildtype and *Mecp2*-mutant mice with either vehicle, vigabatrin (250mg/kg), or SGE-516 (3 mg/kg) daily from weaning through the duration of the study and assessed body weight and disease score weekly, as well as anxiety, motor coordination, learning and memory, and sensorimotor gating at 6-8 weeks of age, and monitored survival (n=15-20 per genotype/drug). We similarly treated female *Mecp2*-heterozygous mice (n=15-20 per genotype/drug) and assessed the same measures at 9-10 weeks and 25 weeks of age. While vigabatrin prevented weight gain in female *Mecp2*-heterozygous mice, neither therapeutic resulted in any survival or behavioral benefit to either male or female *Mecp2* mutant mice. This finding, as well as recent work from our lab showing that reexpression of *Mecp2* solely in glutamatergic neurons also confers significant benefit to *Mecp2* mutant mice, suggests that enhancement of inhibition alone (or excitation alone for that matter) may not be sufficient for reversal of Rett-like phenotypes. We are currently investigating the effect of modulation of both excitation and inhibition using DREADD technology to better understand the

interplay between these two systems in Rett syndrome. The results of these experiments will better direct future therapeutic choices in the search for effective interventions for Rett patients.

Disclosures: **K.M. Ure:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Sage Therapeutics, Lundbeck. **H. Zoghbi:** None.

Poster

496. Rett Syndrome

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Topic: A.07. Developmental Disorders

Title: Longitudinal study of female Rett syndrome mice: touchscreen testing to characterize the onset and progression of cognitive disability

Authors: *L. A. ROTHBLAT, H. L. H. RUTZ;
Psychology, George Washington Univ., Washington, DC

Abstract: Rett syndrome (RTT) is a monogenic neurodevelopmental disorder that in females leads to life-long intellectual disability. RTT is most often caused by spontaneous mutations in the gene that encodes methyl-CpG-binding protein 2 (MeCP2). Much of what is now known about the brain pathophysiology in RTT is attributable to work with transgenic and knockout mice where *Mecp2* is functionally altered. These mice display many cellular and behavioral symptoms that mirror the human condition. Recent studies with RTT mouse models indicate that it may be possible to correct both circuit and neurological dysfunction in young or even adult animals. However, whether a primary clinical feature of the human disorder, cognitive disability, is a significant component of the mouse phenotype and, if so, whether it is correctable, is currently unknown. To study these questions, we have been using a repeating visual reversal touchscreen task to characterize the neurocognitive profile of heterozygous females with two different mutations, *Mecp2 T158A/+* and *Mecp2+/-*. Daily testing began when the mice were 10 weeks of age and is continuing through adulthood. We have found that even mice with unambiguous motor symptoms (reduced locomotor activity in home cage, bilateral hind limb clasp) can be readily tested in the touchscreen. Neither *Mecp2 T158A/+*, nor *Mecp2+/-*, were significantly impaired on acquisition of the initial visual discrimination. In marked contrast to *Mecp2 T158A/+*, *Mecp2+/-* mice required significantly more trials and made significantly more errors when learning the first visual reversal. *Mecp2+/-*, but not *Mecp2 T158A/+*, continue to display a significant impairment on repeated discrimination reversals (testing to 32 weeks of age). We do not yet know if cognitive disability will become evident in *Mecp2 T158A/+* and worsen in *Mecp2+/-* as the mice get older since there is evidence for an enhanced role of MeCP2

in the aging brain. Our findings do show that different *Mecp2* mutations produce distinct cognitive phenotypes that can be accurately tracked in individual animals through longitudinal testing. Moreover, they illustrate how cognitive outcome measures that extend over time could be particularly useful for evaluating possible therapeutic interventions for preventing or reversing intellectual disability in RTT and other autism-related neurodevelopmental disorders.

Disclosures: L.A. Rothblat: None. **H.L.H. Rutz:** None.

Poster

496. Rett Syndrome

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Topic: A.07. Developmental Disorders

Support: NIH Grant RO1NS057398

Title: Symptom reversal during activation of the medial prefrontal cortex in a mouse model of Rett syndrome.

Authors: C. J. HOWELL[1], M. SCENIAK[2], *D. M. KATZ[1];
[1]Dept Neurosciences, Case Western Reserve Univ., Cleveland, OH; [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Rett Syndrome (RTT), a severe neurodevelopmental disorder caused by loss-of-function mutations in the *MECP2* gene, is characterized by postnatal neurological regression leading to severe deficits in motor function, verbal communication and respiratory and autonomic control, among other symptoms. Cardiorespiratory dysfunction severely impacts quality of life and is thought to contribute to early death in some patients (*Kerr, et al., 1997*). Studies of respiratory abnormalities in preclinical models have generally focused on dysfunction in brainstem circuitry, despite the fact that restoring wildtype expression of *Mecp2* in the brainstem of *Mecp2* mutants does not completely restore normal respiration (*Ward et. al., 2011*). Furthermore, behavioral state is known to strongly influence respiratory dysfunction in RTT and *Mecp2* mutants (*Kron et al., 2014)*, suggesting that cortical influences on breathing are abnormal. This hypothesis is supported by recent findings from our laboratory that the medial prefrontal cortex (mPFC), a critical region for cortical modulation of breathing, is hypoactive in *Mecp2* mutants (*Sceniak et al., 2015*). In contrast, *Mecp2* mutants exhibit increased activity in brainstem respiratory nuclei that receive direct projections from the mPFC, including the nucleus tractus solitarius (nTS; *Kron et. al., 2012*). Together, these data suggest that reduced cortical input may contribute to abnormal brainstem function and respiratory abnormalities in RTT. To

test this hypothesis, we took a pharmacogenetic approach to determine whether or not activation of the mPFC in heterozygous female *Mecp2* mutants (Hets) with an excitatory DREADD (Designer Receptor Exclusively Activated by Designer Drugs) would ameliorate respiratory dysfunction. Our findings demonstrate that DREADD activation of mPFC pyramidal neurons that project directly to brainstem respiratory nuclei eliminates apneic breathing in *Mecp2* Hets. Using expression of the immediate early gene product Fos as a surrogate marker of neuronal activity, we find that DREADD activation of the mPFC restores wildtype Fos levels in the mutant nTS. These data indicate that activation of the mPFC reduces brainstem hyperactivity and reverses apneic breathing in *Mecp2* Hets, suggesting that impaired cortical modulation of brainstem respiratory nuclei may contribute to respiratory dysfunction in RTT. Current studies are focused on the role of mPFC circuit dysfunction in other features of the RTT phenotype. This work is supported by NINDS (R01/R56NS057398 to DMK).

Disclosures: **C.J. Howell:** None. **M. Sceniak:** None. **D.M. Katz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Katz is a founding advisor to ArRETT Neurosciences, a company devoted to developing treatments for Rett syndrome.

Poster

496. Rett Syndrome

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SFARI (Simons Foundation Autism Research Initiative)

Rett Syndrome Research Trust

NIMH Silvio Conte Center (P50MH094271)

Title: Rescuing Rett Syndrome through the choroid plexus

Authors: *A. PATRIZI[1], C. LI[1], Y. ZHANG[1], M. FAGIOLINI[1], T. K. HENSCH[1,2]; [1]Neurol., Boston Children's Hosp. Harvard Med. Sch., Boston, MA; [2]Dept. of Mol. and Cell. Biol., Harvard Univ., Cambridge, MA

Abstract: Parvalbumin-positive (PV+) fast-spiking basket cell maturation controls critical periods of brain development and is typically altered across many neurodevelopmental disorders.

For example, PV+ circuits are hyper-connected in the *Mecp2* knockout (KO) mouse model of Rett Syndrome (Durand, Patrizi et al, 2012; Krishnan et al, 2015). This is detected as early as eye-opening and may contribute to the silencing of cortical circuits in *Mecp2* mutants. Here, we first confirmed an intensified PV+ 'gate' in layer 4 of human *post mortem* visual cortex from Rett patients as compared to neurotypical controls. To identify potential factors underlying such PV+ hyper-maturation, we focused on the Otx2 homeoprotein, a potent non-cell autonomous regulator of PV+ cell maturation and maintenance (Sugiyama et al, 2008). Notably, the choroid plexus (ChP) is a major source of Otx2 in the postnatal brain globally impacting PV+ cell state from a distance (Spatazza et al, 2013). We found a striking increase in Otx2 transcription and production in the ChP of *Mecp2* KO mice as compared to wild type littermates. Two genetic strategies were employed to down-regulate Otx2 synthesis. *Mecp2* KO mice crossed to a conditional Otx2 line (*Otx2* fl/fl) were 1) further crossed to a ChP-specific Cre line (*FoxJ1*-Cre), or 2) injected with virus (AAV-Cre) intracerebroventricularly. Both manipulations produced a selective reduction of Otx2 in the ChP without affecting other known sources (retina, superior colliculus). Notably, both manipulations doubled the lifespan and improved the physical appearance and motor performance of *Mecp2* KO mice. Cortical organization of the rescued *Mecp2* KO mice revealed normal cortical thickness and PV+ intensity, fewer Otx2+ cells and normal protein levels. Finally, local disruption of Otx2 transfer into cortical PV cells by short, interfering peptides rapidly reduced Otx2 and PV expression followed by a gradual renormalization of peri-somatic axon bouton number in *Mecp2* KO mice over a 5 to 15-day time course. Taken together, these results suggest down-regulation of ChP-derived Otx2 as a potential therapeutic route for correcting cortical PV-circuit connectopathies and improving several key aspects of the Rett syndrome phenotype.

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Poster

496. Rett Syndrome

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Support: NIH/OD DP5OD009134

NIH/NICHD U54HD083092

NIH/NICHD R01HD062553

Title: Normal phenotypic outcome in a molecular hypomorphic allele of Mecp2 may define the therapeutic window for intervention in MeCP2 disorders

Authors: *C. S. WARD[1,2], S. SORIANO[1], M. R. PITCHER[3], A. CHAHROUR[1], A. J. LIANG[1], C. M. MCGRAW[4], J. L. NEUL[2], R. C. SAMACO[1];
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Abstract: Rett syndrome (RTT) is caused by loss-of-function mutations in the gene encoding the transcriptional modulator *Methyl-CpG-Binding Protein 2* (*MECP2*); in contrast, chromosomal duplications spanning *MECP2* result in a progressive neurological disorder. The human conditions and their respective mouse models support the notion that the levels of MeCP2 must be tightly regulated for normal neurological function. Furthermore, previous studies demonstrated several neurobehavioral abnormalities in mice with a 50% reduction in MeCP2 expression. These studies raise the concern that the therapeutic window for intervention with respect to modulating MeCP2 levels may be narrow. Therapies that surpass the upper bound of this window and result in excess MeCP2 may induce disease, whereas therapies that fail to reach the lower bound of this window may not improve disease. We therefore set out to test the neurobehavioral and molecular consequences in a mouse model that has an approximate 20% reduction in MeCP2 levels to determine whether complete restoration of MeCP2 expression is required for normal phenotypic outcome. We found that mice harboring a mild hypomorphic allele of MeCP2 are comparable to wild-type animals in a broad range of behavioral, physiological and molecular measurements. Furthermore, we found that the 20% reduction in this mouse model reflects an overall decrease in MeCP2 expression in both neurons and glia. These data provide a genetic proof-of-concept that absolute normalization of MeCP2 levels may not be necessary for normal neurological function, and suggest that increasing MeCP2 levels to at least 80% of the normal range may be beneficial for RTT.

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Poster

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Support: RSRT (401 Project)

Title: Optimization of an antisense oligonucleotide therapy in humanized MECP2 duplication mice and human neurons

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Abstract: *MECP2* duplication syndrome is one of the most common genomic rearrangements in males and is characterized by autism, intellectual disability, motor dysfunction, anxiety, epilepsy, recurrent respiratory tract infections and early death. Our classic *MECP2* duplication syndrome mouse model (*MECP2*-TG) expresses a human *MECP2* allele in addition to the endogenous mouse *Mecp2* allele, and recapitulates many of the characteristics of the disease at the molecular, electrophysiological, and behavioral levels. We recently showed that treatment with an antisense oligonucleotide (ASO) that targets *MECP2* induces a broad phenotypic rescue in adult symptomatic *MECP2*-TG mice. Antisense oligonucleotides are small, modified nucleic acids that can selectively hybridize to messenger RNA transcribed from a target gene to silence it. Because *MECP2*-ASOs are human-specific and only target human *MECP2* mRNA, one is assured in this model with one human and one mouse copy that ASO treatment will leave the expected 1X levels from the mouse allele. In humans, however, the two copies are identical and one must ensure that the ASO is titrated to target the human allele such that MeCP2 levels go from 2X to 1X. To advance the ASOs approach we must establish models of *MECP2* duplication syndrome that precisely mimic the human condition by having two human *MECP2* alleles and no mouse allele. We therefore generated and characterized a humanized model of the *MECP2* duplication syndrome by breeding *MECP2*-TG mice with *MECP2*-GFP mice (*MECP2* tagged with GFP at the C-terminus), on an endogenous *Mecp2*-null background. These humanized *MECP2* duplication mice are viable, have no apparent physical abnormalities, overexpress MeCP2 by 2-fold, and display properties reminiscent of *MECP2* duplication syndrome. In addition, we generated and validated iPSCs-derived neurons from *MECP2* duplication patients. We are currently studying the pharmacokinetics of the *MECP2*-ASOs and the safety margin of MeCP2 levels in both the humanized mouse model as well as in the iPSCs-derived neurons.

Disclosures: E. Sztainberg: None. **Y. Shao:** None. **M. Zaghlula:** None. **F. Rigo:** A. Employment/Salary (full or part-time): Inois Pharmaceuticals. **H.Y. Zoghbi:** None.

Poster

496. Rett Syndrome

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 496.13/F11

Topic: A.07. Developmental Disorders

Title: Modulating elevated brain VEGF-A to treat Fragile X Syndrome vocalization deficits

Authors: *A. BELAGODU, A. JOHNSON, R. GALVEZ;
Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Fragile X Syndrome (FXS) is the leading form of inherited mental retardation. It is caused by the transcriptional silencing of fmr1, the gene which codes for the fragile X mental retardation protein. FXS patients have been shown to exhibit numerous behavioral and cognitive impairments, such as ADHD, OCD, and autistic-like behavior. In addition to these behavioral abnormalities, they also exhibit various deficits in speech and language. Studies in human FXS patients have shown that they form abnormal sentence structures, have issues with utterances, increased repetition of sounds and words, and articulation. To study the biological underpinnings of these speech abnormalities, our prior study used a mating separation procedure to conduct a detailed spectral analysis of FXS mouse ultrasonic vocalizations (USVs) and demonstrated similar repetitive vocalization abnormalities, strengthening the neuroethological relevance of this model. Interestingly our prior studies have demonstrated that FXS mice also have elevated brain VEGF-A levels; that when blocked can alleviate neuronal and cognitive abnormalities. The current study set out to determine if decreasing VEGF-A binding to its receptor can alleviate FXS USV abnormalities. In this study, a standard mating separation procedure was used. Individual adult male mice were briefly exposed to an adult female mouse. Once the male expressed an interest in the female, the female was removed which elicited USVs from the male. The male USVs were recorded using an ultrasonic recording system (Avisoft, Germany) and analyzed using an existing custom MatLab program designed to detect, spectrally analyze, and classify mouse USVs. Following the exposure, male mice underwent a series of injections of bevacizumab, a VEGF-A antagonist, every other day for 10 days. The day after the fifth injection, the same aforementioned procedure was used, and the USVs generated were compared to those obtained from the mouse prior to injections. Pretreatment analyses of USV properties demonstrated similar FXS abnormalities as previously reported. However initial analysis has indicated that following blocking of VEGF-A binding to its receptor FXS mice exhibited USV vocalizations more similar to those observed in controls. These findings suggest that modulating VEGF-A levels can have a global effect and potentially help alleviate both cognitive and behavioral FXS deficits

Disclosures: **A. Belagodu:** None. **A. Johnson:** None. **R. Galvez:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 497.01/F12

Topic: A.07. Developmental Disorders

Support: NIH Grant AA017978

Title: Confirmation of thyroxine treatment for fetal alcohol spectrum disorders in a vulnerable breed

Authors: *E. TUNC-OZCAN, K. M. HARPER, B. M. ANDRUS, E. E. REDEI;
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Abstract: Fetal Alcohol Spectrum Disorder (FASD) encompasses a continuum of disabilities caused by fetal alcohol exposure (FAE). Hippocampus-based cognitive and affective behavioral deficits are among the most debilitating consequences of FAE. In our rat model, offspring exposed to ethanol (E) *in utero* recapitulates these deficits of FASD. Similar deficits, particularly in the cognitive domain, are found in children of mothers with subclinical hypothyroidism during pregnancy. Since E-consuming women and also the rat dam show decreased levels of thyroxin (T4), we administered T4 into the E-containing diet aiming to reverse the deleterious effects of E-induced maternal hypothyroidism. In two separate studies hippocampus-dependent behavioral deficits were reversed by T4 supplementation to the E-consuming Sprague-Dawley (S) rat dams. Furthermore, the deleterious effects of FAE transmitted to the next generation through the matrilineal lineage were also prevented by T4 administration to the E-consuming S grand-dams. In both studies, adult FAE offspring of the S strain showed a hyperthyroid profile with elevated plasma free T3 and decreased plasma TSH levels, which were normalized by maternal T4. Hippocampal expression of the imprinted *Dio3* gene, which metabolizes the biologically active thyroid hormone, were elevated in the FAE S offspring, and in the progeny of FAE S females mated with a Brown Norway (B) male. *Dio3* transcript levels were also normalized by T4 administration to the E-consuming dam or grand-dam, respectively. In the present study, S females were mated with B males and the S dams received control diets (*ad libitum* and nutritional control) or E containing liquid diet with and without T4 (0.3mg/l diet) as in the previous studies. Adult SB offspring were tested for hippocampus-dependent contextual fear conditioning, despair-like behavior in the forced swim test, thyroid function and hippocampal expression of *Dio3* and the thyroid hormone-regulated neurogranin *(Nrgn)* gene.
Both decreased fear memory and increased depression-like behavior were reversed by maternal T4 administration in the FAE SB offspring. Hippocampal *Dio3* and *Nrgn* expression showed changes in response to FAE and T4 treatment similar to those in the previous two studies, despite the hypothyroid peripheral thyroid profile; opposite to those observed previously. Thus, *Dio3*,

rather than peripheral thyroid function may regulate brain thyroid hormone availability. Maternal T4 administration reproducibly reverses cognitive and affective behavioral deficits induced by FAE, likely via regulating brain *Dio3* levels.

Disclosures: E. Tunc-ozcan: None. **K.M. Harper:** None. **B.M. Andrus:** None. **E.E. Redei:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 497.02/F13

Topic: A.07. Developmental Disorders

Support: NSF Graduate Research Fellowship

Title: Choline supplementation ameliorates brain and behavior phenotypes associated with prenatal ethanol exposure in a mouse model of FASD

Authors: *R. T. BOTTOM[1], C. W. ABBOTT, III[2], J. A. RUMSCHLAG[1], K. J. HUFFMAN[1]; [1]Neurosci., Univ. of California, Riverside, Riverside, CA; [2]Dept. of Genet. and Sch. of Med., Stanford Univ., Palo Alto, CA

Abstract: Fetal alcohol spectrum disorders, or FASD, broadly describe the spectrum of deleterious effects resulting from prenatal exposure to ethanol (PrEE). Despite widespread warnings, CDC recently reported that nearly 20% of pregnant women age 34-40 admitted to drinking during their pregnancies. Thus, we must look beyond abstinence to new preventative, therapeutic approaches. Recently, choline supplementation has been reported to improve both behavioral (Thomas et al., 2010) and neurological (Bekdash et al., 2013) deficits in PrEE rats. Here, we demonstrate that maternal choline supplementation administered with ethanol reduces genetic, neuroanatomical, behavioral defects associated with PrEE. Abnormal development of intraneocortical connections, neuroanatomical structures, and ectopic neocortical gene expression are previously documented phenotypes in newborn PrEE offspring (El Shawa et al., 2013; Abbott et al., 2016). These effects are ameliorated in PrEE newborn mice supplemented with choline via maternal self-administration throughout gestation. Finally, we demonstrate that PrEE mice supplemented with choline show a reduction in sensorimotor deficits associated with prenatal exposure to ethanol as well as significant improvements in anxiety and depression-like behaviors, as assessed by a battery of behavioral tests at postnatal day 20. These data suggest choline supplementation may be able to reverse the development of neurological and behavioral deficits in PrEE mice. These results implicate choline as a possible clinical prevention for

women who continue to drink during their pregnancies, thus reducing the incidence or severity of FASD in humans.

Disclosures: **R.T. Bottom:** None. **C.W. Abbott:** None. **J.A. Rumschlag:** None. **K.J. Huffman:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 497.03/F14

Topic: A.07. Developmental Disorders

Title: Developmental vitamin D deficiency ameliorates the effects of prenatal exposure to ethanol

Authors: *M. C. SANCHEZ VEGA[1], S. CHONG[2], T. H. J. BURNE[1];
[1]The Queensland Brain Inst., Brisbane, Australia; [2]Mater Res. Institute- The Univ. of Queensland, Brisbane, Australia

Abstract: There is a high prevalence of vitamin D deficiency and exposure to low levels of alcohol consumption in pregnant women (Bodnar et al., 2007, Daly et al., 2012). However, there are a paucity of studies that have addressed the impact of both of these exposures on the offspring's vulnerability to neuropsychological disorders later in life. The aim of this study was to examine whether the absence of vitamin D during gestation in mice would alter the effects of prenatal exposure to low dose ethanol.
Four-week old female C57Bl/6J and Balb/c mice were placed on a vitamin D deficient or standard diet for 6 weeks and mated at 10 weeks of age. Females were exposed to either 10 %(v/v) ethanol or water for GD 0-8 and offered water for the rest of gestation. Analyses included ultrasonic vocalizations at P7, social interaction (SI) at P21, locomotion and spatial learning and memory at P70. Ex-vivo MRI and Next-generation sequencing (NGS) was carried out in neonatal brains, followed up by gene and protein expression analyses on candidates in P0 and P70 tissue.
The main findings at P7-21 were increased calling rate in prenatal ethanol-exposed (PEE) males, altered distribution of call types among DVD and EtOH groups and impaired SI in PEE males. Hypolocomotion was encountered in PEE adult males, while learning and memory performance in the Active Place Avoidance task was unaffected in all Groups. Gross brain anatomy was unaffected by DVD deficiency and PEE. RNA sequencing data showed that neonatal male offspring were more vulnerable to the effects of DVD and PEE, showing eight dysregulated genes relevant in hippocampal development. Of particular interest, Grin3b is a protein-coding

gene that was downregulated in DVD-PEE males, which encodes an ionotropic glutamate receptor, is expressed in the hippocampus and studies in mice have shown that mice homozygous for a null allele present a behavioral phenotype including mild motor impairment, hypolocomotion and impaired social interaction in a novel environment. Vglut2 was downregulated in PEE adult male hippocampus. DVD-PEE animals showed no difference in Vglut2 expression compared to controls.
We conclude from the present results that DVD deficiency does not increase offspring vulnerability to PEE; on the contrary, the combination of exposures either reversed the phenotype observed with a single exposure or had no effect.
We are currently investigating ultrastructural changes in ventricles and hippocampus at P0, based on previous individual findings on DVD and PEE. We are also investigating the expression of candidate genes obtained from the NGS study to evaluate possible protective mechanisms of DVD deficiency on exposure to ethanol.

Disclosures: M.C. Sanchez Vega: None. **S. Chong:** None. **T.H.J. Burne:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 497.04/F15

Topic: A.07. Developmental Disorders

Support: NIH 1R15DA034912-01A1

Title: Behavioral and plasticity mechanisms of the associative effects of nicotine in the neonatal quinpirole model of schizophrenia

Authors: *A. DENTON[1], S. L. KIRBY[2], C. L. KAESTNER[3], K. C. BURGESS[3], J. D. WHERRY[3], J. M. DOSE[4], R. W. BROWN[3];
[2]Psychology, [3]Biomed. Sci., [1]East Tennessee State Univ., Johnson City, TN; [4]Psychology, St. Norbert Col., De Pere, WI

Abstract: Schizophrenics are significantly more likely to smoke cigarettes than the general population. In Experiment 1, we analyzed the effects of a rewarding versus an aversive dose of nicotine using the neonatal quinpirole (QUIN; dopamine D2/D3 agonist) model of schizophrenia. In Experiment 2, we examined the effects of antipsychotic treatment upon the associative reward of nicotine within this same model. Neonatal QUIN treatment to rats results in increased dopamine D2 receptor sensitivity throughout the rat's lifetime, consistent with schizophrenia. Rats were neonatally treated with QUIN (1 mg/kg dose) or saline from postnatal days (P)1-21.

Animals were then raised to P41 without any further drug treatment. Subjects were administered two consecutive pre-conditioning drug free preference tests in a three chamber shuttle box on P41 and P42 to determine initial preference. In Experiment 1, beginning on P43, animals were conditioned with saline, a 0.6 mg/kg or a 1.8 mg/kg free base dose of nicotine for eight consecutive days. A drug free post-conditioning preference test was given on P51. Approximately 24 h following the post-conditioning test, brain tissue was harvested and analyzed for mammalian target of rapamycin (mTOR) and phosphorylated-CREB (pCREB) in the nucleus accumbens. In Experiment 2, animals were treated identically as in Experiment 1, but were conditioned with nicotine which was preceded by an injection of either a typical antipsychotic (haloperidol, 0.5 mg/kg dose) or an atypical antipsychotic (clozapine, 2.5 mg/kg dose) for a period of eight days which was followed by a drug free preference test. In both experiments, the difference between time spent in the paired context between pre-test and post-test was utilized as a measure of associative reward. Results revealed that neonatal QUIN enhanced the rewarding effects of nicotine, but neutralized the aversive effects compared to controls. Neonatal QUIN also significantly decreased accumbal mTOR and pCREB results will be presented. In Experiment 2, we found that treatment with clozapine reduced the enhancement of nicotine conditioned place preference, but haloperidol completely reduced nicotine place preference to control levels. These findings show that neonatal QUIN enhances the rewarding associative effects of nicotine, and that the typical antipsychotic haloperidol was more effective at eliminating the associative rewarding effects of nicotine likely due to its potent D2 antagonistic effects.

Disclosures: **A. Denton:** None. **S.L. Kirby:** None. **C.L. Kaestner:** None. **K.C. Burgess:** None. **J.D. Wherry:** None. **J.M. Dose:** None. **R.W. Brown:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

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Program#/Poster#: 497.05/F16

Topic: A.07. Developmental Disorders

Support: NIH 1R15DA034912-01A1

Title: Prepulse inhibition deficits in the neonatal quinpirole model of schizophrenia: Epigenetic evidence and sex differences

Authors: W. D. GILL, J. D. WHERRY, K. C. BURGESS, *R. W. BROWN;
East Tennessee State Univ. Dept. of Biomed. Sci., Johnson City, TN

Abstract: Neonatal quinpirole (QUIN; dopamine D2/D3 agonist) administered from postnatal days (P)1-21 results in an increase of dopamine D2 receptor sensitivity, similar to schizophrenia and is now an established rodent model of schizophrenia. The day after birth, male and female Sprague-Dawley rats were given a daily 1 mg/kg injection of either QUIN or saline from P1-21. One subset of these animals were behaviorally tested on PPI, referred to as first generation (F0). A different subset of animals were allowed to reach adult age (P60) and female and male QUIN-treated pairs from separate litters were bred. Their offspring were also used as subjects (F1 generation). Prepulse inhibition (PPI) is a measure of sensorimotor gating reduced in individuals diagnosed with schizophrenia. Trial types were defined as prepulse trials (73, 76, 82dB), startle stimuli trials (120 dB), or no stimulus (70 dB white noise; no prepulse or pulse). Animals were tested for six consecutive days and given an ip saline injection 10 min before testing, followed by testing for another six consecutive days and given an ip nicotine (0.5 mg/kg free base) or saline injection 10 min before testing. PPI testing for F0 generation animals occurred between P35-46, and testing for F1 generation animals occurred between P44-55. In one subset of generation F1 animals, rats were ip injected with a 0.1 mg/kg dose of quinpirole and immediately observed for 60 min and the number of yawns were recorded at P60. Yawning is a behavioral event mediated by the dopamine D2 receptor. Results revealed that neonatal QUIN resulted in PPI deficits throughout the six days of testing in the F0 generation regardless of the prepulse stimulus, but females demonstrated a less robust PPI deficit as compared to males. Nicotine given during the final 6 days of testing partially alleviated the PPI deficits in both males and females. The F1 generation also demonstrated PPI deficits, but the impairment was only in males and dissipated by day 6. Nicotine did not affect PPI in these animals. Finally, F1 generation rats demonstrated a robust increase in yawning compared to controls, demonstrating an increase in D2 receptor sensitivity. Brain tissue is being analyzed for changes in the dopamine D2 receptor signaling pathway.

Disclosures: **W.D. Gill:** None. **J.D. Wherry:** None. **K.C. Burgess:** None. **R.W. Brown:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

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Topic: A.07. Developmental Disorders

Support: China ZJU 181110-193544B01/007

MOST103-2320-B-010-014-MY2

MOST103-2321-B-010-016

MOST 102-2221-E-010-011-MY3

Title: Deep brain stimulation overcomes cognitive deficits in autism rat: fMRI evaluation the improvement of symptoms

Authors: *H.-Y. LAI[1], S.-J. LI[2], H.-Y. WANG[2], H.-F. WU[3], P. CHEN[4,5], Y.-Y. CHEN[2], H.-C. LIN[3];
[1]Interdisciplinary Inst. of Neurosci. and Technol., Zhejiang Univ., Zhejiang Province, China; [2]Inst. of Biomed. Engin., Natl. Yang-Ming Univ., Taipei, Taiwan; [3]Natl. Yang-Ming Univ., Department and Institute of Physiology, Taiwan; [4]Dept. of Psychiatry, Natl. Cheng Kung Univ. Hosp., Tainan, Taiwan; [5]Col. of Med., Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder. The pathogen of autism has been unknown till now, researchers suspected genetic factors as the most likely cause and then be triggered by environmental factor. Valproic acid (VPA) is a well know antiepileptic drugs and thought to alter mood stability like modifying gamma-aminobutyric acid (GABA) levels. The previous literatures illustrated that rats induced by VPA has been seen as animal model of autism. VPA-induced ASD rats change the pattern of dendritic development, but don't affect neural growth or stunt in motor cortex in some studies. VPA can be viewed as an inhibitor of connectivity in motor circuits. Deep brain stimulation (DBS) is considered to be a method for local stimulation, which modulates neural function of broader networks. Consideration of the use of thalamic electrical stimulation to treat patients with severe disorders of consciousness has a long history. In this study, we proposed the central thalamic DBS (CL-DBS) as a potential therapy for VPA-induced ASD rat model. We used the resting-state functional MRI (rsfMRI) and a standard behavioral social testing to evaluate the changes of the functional connectivity in cortico-striatal circuits and the social behavior, respectively. The social testing included three chambered facility, empty, central, and social, and how long the rats spent in each facility were calculated. Results showed that CL-DBS could increase the correlation between primary motor cortex (M1) and stratum (Str), as well as the social behavior of rats could indeed be improved. This study demonstrates that CL-DBS is an effective method to treat the VPA-induced ASD rats.

Disclosures: H. Lai: None. **S. Li:** None. **H. Wang:** None. **H. Wu:** None. **P. Chen:** None. **Y. Chen:** None. **H. Lin:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

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Topic: A.07. Developmental Disorders

Support: UofL Undergraduate Research Scholar award to EB & EPC

UofL Dept Biol award to EG

Graduate Student Council Research award to EG

Outstanding Neuroscience Trainee award from Louisville SFN Chapter to EG

Title: Evaluating sex differences and the effect of perinatal testosterone in the VPA mouse model of autism

Authors: E. GORDON[1], E. BRYAN[1], M. D. COLLINS[1], S. DUGAN[1], D. DUVALL[1], K. HAMILTON[1], A. JACOB[1], T. KARIM[1], O. OBRIK-ULOHO[1], E. PENA-CALDERIN[1], *C. CORBITT[2];
[1]Biol., [2]Univ. of Louisville, Louisville, KY

Abstract: Autism spectrum disorder (ASD) is a behaviorally-defined condition with a wide range of symptoms, severity, and co-morbidities. The "multiple-hit hypothesis" states that a combination of genetic predisposition and environmental insults increase autism risk. Autism is more common in males than females, pointing to 'maleness' as a potential 'hit', but the mechanism of this bias remains unknown, including whether the presence of fetal testosterone (fT) alone is responsible for the increased ASD risk or if other factors contribute to this bias. Evidence suggests that elevated levels of fT, which is responsible for male-typical brain development, are present in those later diagnosed with ASD. Valproic acid (VPA), an antiepileptic medication, increases ASD risk in humans when used during pregnancy. This drug has been used to develop an animal model of ASD in rodents, which mimics many of the features of ASD, both behavioral (e.g., social deficits, increased repetitive behaviors, deficits in communication) and morphological (e.g., reductions in Purkinje cell number and cerebellar volume). Male rodents are more vulnerable to some VPA effects, making this an appropriate model to study sex differences in ASD. This project uses a mouse model of ASD to mimic differential susceptibility of males and females to a prenatal insult (VPA), and to determine whether masculinizing females perinatally with testosterone propionate (TP) following prenatal VPA treatment will also masculinize their risk for abnormalities in brain development and behavior relevant to ASD. Our study focuses on cerebellar morphology and behaviors applicable to this brain region because it is often affected in ASD, as well as in the VPA animal model. Our results support other research that has indicated VPA treatment causes motor development delays. Further analysis will provide insight into abnormalities in neurodevelopment and behavior caused by VPA treatment alone, if/how these abnormalities vary by sex, and how perinatal TP affects the severity of these abnormalities in each sex.

Disclosures: **E. Gordon:** None. **E. Bryan:** None. **M.D. Collins:** None. **S. Dugan:** None. **D. Duvall:** None. **K. Hamilton:** None. **A. Jacob:** None. **T. Karim:** None. **O. Obrik-Uloho:** None. **E. Pena-Calderin:** None. **C. Corbitt:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

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Program#/Poster#: 497.08/F19

Topic: A.07. Developmental Disorders

Support: NIH Grant AA12435

Title: Effects of environmental enrichment in a valporic acid model of autism in rats

Authors: *C. D. MARTIN[1,2], A. M. GEORGE[2], K. ISHIWARI[2], S. HAJ-DAHMANE[2], R. Y. SHEN[2], L. W. HAWK, Jr.[1], J. B. RICHARDS[2];
[1]Psychology, Univ. at Buffalo Dept. of Psychology, Buffalo, NY; [2]Res. Inst. on Addictions, Buffalo, NY

Abstract: Autism spectrum disorder (ASD) is a behavioral disorder characterized by dysfunction in formation and maintenance of social relationships, in communication, and repetitive/stereotyped behaviors and hyper-reactivity to sensory stimulation. Valporic acid (VPA), an anticonvulsant medication, has been shown to increase the risk of autism in children whose mothers have a history of VPA use. It has become a popular tool for producing a behavioral phenotype consistent with an ASD diagnosis in a variety of non-human animal species. Some pathologies (such as ASD) modeled in animals have been reversed or ameliorated under environmentally complex housing conditions. Typical experimental paradigms assess play behaviors and repetitive behaviors to test effects of VPA on social dysfunction and stereotypy; operant reinforcement paradigms are used less frequently. Experiments that do employ operant designs use only one reinforcer at a time, unlike the natural environment where multiple reinforcers are available simultaneously. This experiment tested 84 Sprague-Dawley rats in a 2x2 design, assessing the effects of a pre-natal VPA manipulation and an environmental enrichment manipulation in a testing chamber with access to different forms of reinforcement. Animals were tested across nine days; a three-day pre-exposure period during which there were no programmed reinforcers, followed by three days where a sensory stimulus (light onset) was available on a VI 1 minute interval schedule, and finally a three-day period where a social stimulus (access to a cage-mate) was available concurrent with the sensory stimulus. A multifactorial analysis was conducted to assess the effects of the manipulation on absolute responding, relative rate of responding, habituation of responding, and contact time, across all three testing conditions. Introduction of the response contingent light stimulus increased responding proportionately more for VPA-treated animals than controls; enrichment did not affect light responding. Upon introduction of the social reinforcer on the opposite side of the test apparatus, enrichment increased relative preference but not absolute responding for the social side; VPA had no effect. The two manipulations did not interact. The current model of VPA-

induced autism in rats may lack ecological validity, given that a concurrent reinforcement paradigm appears to decrease the effects of VPA.

Disclosures: **C.D. Martin:** None. **A.M. George:** None. **K. Ishiwari:** None. **S. Haj-Dahmane:** None. **R.Y. Shen:** None. **L.W. Hawk:** None. **J.B. Richards:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

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Program#/Poster#: 497.09/F20

Topic: A.07. Developmental Disorders

Support: R21HD080498

Title: Intranasal oxytocin delivery in juvenile nonhuman primates

Authors: ***T. MURAI**[1,2], C. PHI[1], L. OLSEN[1], S. FREEMAN[1], T. WEINSTEN[1], K. BALES[1], J. CAPITANIO[1], M. PLATT[3], M. BAUMAN[1];
[1]California Natl. Primate Res. Ctr., UC Davis, Davis, CA; [2]Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan; [3]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Converging evidence from clinical and preclinical research indicates that oxytocin (OT) plays a key role in species-typical social behavior. Although OT-therapy is being investigated as a safe and promising therapy for autism spectrum disorder (ASD), we know very little about the efficacy, safety, and neurobiological mechanisms associated with OT exposure. Preclinical research utilizing macaque monkeys has demonstrated that intranasally administered OT can modulate aspects of primate social cognition, including social perception, imitation, and social vigilance. Here we describe efforts to develop an OT delivery system for juvenile macaque monkeys that is compatible with an expanded behavioral test battery that integrates non-invasive eye-tracking and unconstrained social interactions. Four juveniles (2 males and 2 females) were trained to drink a fluid reward from a small tube embedded below a nebulizer that delivered aerosolized OT or saline placebo over the course of 10 minutes. We utilized a series of positive reinforcement training techniques to reach a target mask exposure time of approximately 5 minutes per each 10 minute session. OT doses ranging from 24-48IU were evaluated in pilot studies and cumulative drinking time was quantified for each experiment as an index of OT exposure. To evaluate peripheral OT concentration, blood samples were collected 15, 30, 60 and 90 minutes after OT or saline exposure. Preliminary evaluation of plasma OT concentration following OT exposure indicates that this delivery system is effective for increasing peripheral OT concentrations. Plasma OT concentration will be compared with central OT concentrations

indexed by cerebrospinal fluid assays, and both measures will be used as predictors of behavior. If effective, this novel delivery system for young rhesus monkeys will provide a testbed to evaluate the effects of OT exposure during development on species-typical social interactions and advance efforts to translate basic science OT research into safe and effective OT therapies.

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Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

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Program#/Poster#: 497.10/F21

Topic: A.07. Developmental Disorders

Title: Positive allosteric modulation of nmda receptors ameliorate behavioral and electrophysiological phenotypes in a pre-clinical model of smith-lemli-opitz syndrome.

Authors: ***M. C. LEWIS**, M. QUIRK, R. HAMMOND, C. MACIAG, M. ACKLEY, G. BELFORT, G. MARTINEZ BOTELLA, J. DOHERTY, A. ROBICHAUD;
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Abstract: Smith-Lemli-Opitz Syndrome (SLOS) is a rare genetic disorder caused by mutations in the 7-dehydrocholesterol reductase gene DHCR7, encoding the enzyme that transforms 7-dehydrocholesterol to cholesterol. Loss of function of DHCR7 leads to a reduction in total cholesterol production that contributes to symptoms including microcephaly, moderate to severe intellectual disability, sensory hypersensitivity, stereotyped behaviors, dysmorphic facial features, and syndactyly of the second and third toes, with the severity of clinical presentation being inversely correlated with serum cholesterol levels. Importantly, SLOS patients also have reduced levels of the brain specific cholesterol metabolite 24(S)-hydroxycholesterol (24(S)-HC) due to the reduction in brain cholesterol driven by altered DHCR7 function. As 24(S)-HC is known to be an endogenous positive modulator of the NMDA receptor, it is conceivable that some of the behavioral and cognitive features of SLOS may be driven by NMDA receptor hypofunction. In the current studies, we describe a pharmacological animal model of SLOS induced by chronic administration of the DHCR7 inhibitor AY9944. Chronic inhibition of DHCR7 leads to reductions in cholesterol levels and 24(S)-HC, reduced total brain weight, increased liver weights, deficits in long-term potentiation and paired pulse facilitation, increases in sharp wave discharges, and locomotor hyperactivity, which closely resemble many of the key features of SLOS. Herein, we report that, in the animal model, administration of a positive allosteric modulator of the NMDA receptor, SAGE-718, attenuated AY9944-induced sharp wave

discharges and hyperactivity. These studies provide important data by which to assess proof of concept of the utility of NMDA PAMs in SLOS, and suggest that other cerebrosterol deficit disorders, as well as diseases resulting from NMDA hypofunction, may be areas of interest for further study of this class of compounds.

Disclosures: **M.C. Lewis:** A. Employment/Salary (full or part-time): Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **M. Quirk:** A. Employment/Salary (full or part-time): Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **R. Hammond:** A. Employment/Salary (full or part-time): Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **C. Maciag:** A. Employment/Salary (full or part-time): Sage Therapeutics. **M. Ackley:** A. Employment/Salary (full or part-time): Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **G. Belfort:** A. Employment/Salary (full or part-time): Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **G. Martinez Botella:** A. Employment/Salary (full or part-time): Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **J. Doherty:** A. Employment/Salary (full or part-time): Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **A. Robichaud:** A. Employment/Salary (full or part-time): Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 497.11/F22

Topic: A.07. Developmental Disorders

Support: NEI Grant EY017141

Title: Rescue of a mutant T8993G mouse model of NARP and MILS with mito-targeted AAV9 containing wild type ATP6

Authors: *H. YUAN, H. YU, J. GUY;
Univ. of Miami, Miami, FL

Abstract: To develop an effective gene therapy for the rescue of a human mutant T8993G ATP6 mouse model of NARP and MILS, we created mito-targeted AAV modified VP2 by mitochondria targeting sequence cox8. We synthesized human normal ATP6 gene fused by a flag epitope at the c-terminal with stop codon followed by mitochondrial encoded *mCherry*, which was cloned under the control of the mitochondrial heavy strand promoter (HSP) into the self-complementary AAV backbone (sc-HSP-*ATP6FLAG+mcherry*). ATP6 expression was significantly increased 35 fold in NARP mitochondria infected by mito-targeted AAV9/ATP6. ATP synthesis was increased 41%. NARP cell proliferation significantly increased 49%. 53 of a mutant T8993G ATP6 founder mouse (A6M) was generated by microinjection of mito-targeted AAV2 with sc-HSP-*mutATP6FLAG+mcherry* into mouse zygotes. A6M mice developed severely leigh syndrome, mortality high to 78%. A6M mice were also observed vision loss, paralysis, seizure and hunched severely leigh syndrome phenotype. 10ul of mito-targeted AAV9/ATP6 were respectively injected into 3 month old A6M and 20 month old A6M mice with a genotype and phenotype of T8993G NARP and MILS, and untreated A6M mice of same conditions as controls. After injection of mito-targeted AAV9/ATP6 at 3 month old, treated A6M mice showed 100% survival compared to 30% survival in untreated A6M mice for 18 months. PERG showed amplitudes of treated A6M mice was increased to 19.6 uV after six month compared to 11.3 uV for untreated A6M mice. At later stage A6M mice with hunched, rota rod testing showed latency-to-fall time was significantly increased after injection for two weeks. Video showed the movement with paralysis hind limbs mice prior to their spontaneous deaths were significantly improved after two days of injection of mito-targeted AAV9/ATP6. Our results showed mito-targeted AAV9-wildtype ATP6 can successfully rescue mutant T8993G ATP6 mice significantly reducing mortality rate, improving defective visual function, ataxia and paralysis suggesting it may be helpful for MILS and NARP patients.

Disclosures: **H. Yuan:** None. **H. Yu:** None. **J. Guy:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

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Topic: A.07. Developmental Disorders

Support: Polish National Science Center Grant 2014/15/D/NZ4/04274

Polish National Science Center Grant 2011/03/N/NZ29/05222

European Union Seventh Framework Programme Grant 312097

Title: Pregnancy at advanced maternal age affects behavior and hippocampal gene expression in mouse offspring

Authors: S. SAMPINO[1,2], A. M. STANKIEWICZ[1], F. ZACCHINI[1], J. GOSCIK[3], A. SZOSTAK[1], *A. H. SWIERGIEL[4], J. A. MODLINSKI[1], G. E. PTAK[1,5,2];
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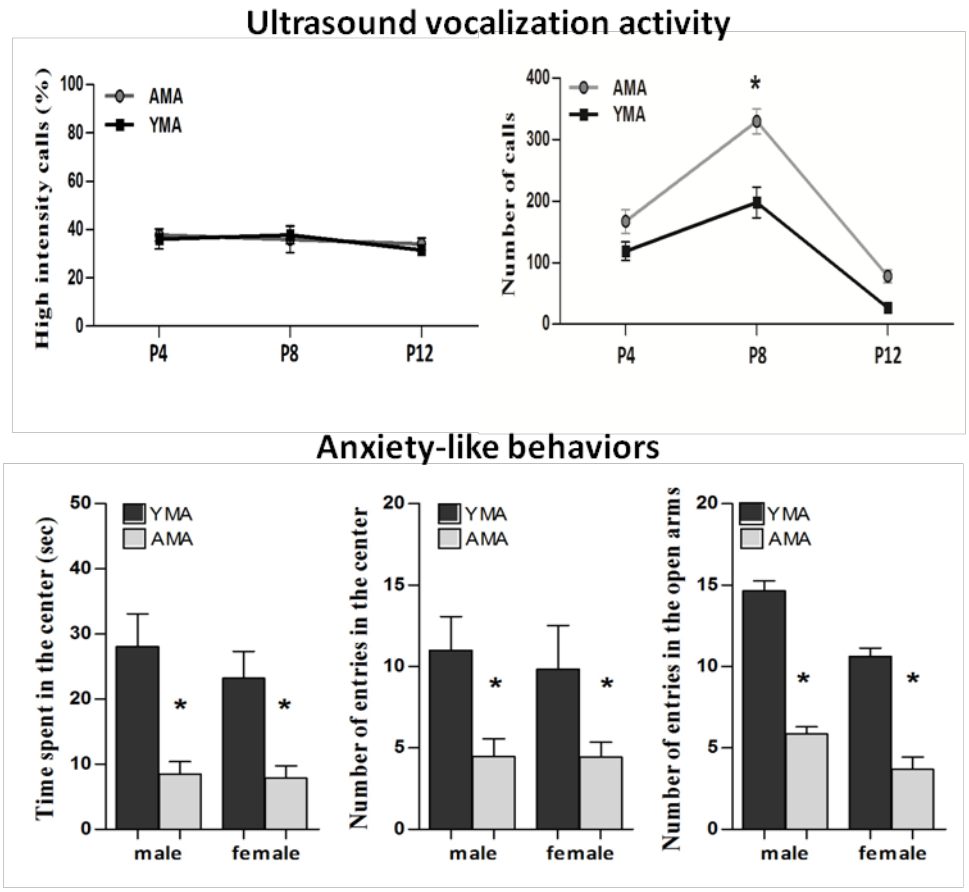
Abstract: Advanced Maternal Age (AMA) is a risk factor for neurological and neuropsychiatric disorders in offspring. We developed a mouse model in order to investigate whether pregnancy at advanced age may evoke behavioral and brain gene expression changes in the offspring. Mice conceived by 15-18 months old (15-18M) or 3M control females were delivered by cesarean section and fostered after birth by 3M dams. Offspring were subjected to a battery of behavioral tests and genome-wide mRNA expression was then analyzed using microarrays in the hippocampus of 4M male offspring. We observed increased ultrasound vocalization activity during juvenile social isolation and increased anxiety-like behaviors in adult offspring conceived by old females (Figure 1). The hippocampal mRNA expression of several genes involved in protein post-translational modification and protein homeostasis was affected by AMA. In conclusion, pregnancy at advanced age yields offspring with abnormal behaviors and altered patterns of gene expression in the hippocampus, resembling human neuropsychiatric and neurodegenerative disorders. The effects had not been reversed by post-natal maternal cares provided by young foster mothers. This suggests that prenatal conditions associated with AMA may negatively affect fetal brain development and hence post-natal behaviors.



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Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

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Topic: A.07. Developmental Disorders

Support: PIP CONICET 00323

UBACYT 20020120100149

CONICET fellowship

UBA fellowship

Title: Age-dependent changes might be induced in a rat hippocampal oxidative marker after noise exposure. Potential restoration after rearing in an enriched environment

Authors: S. J. MOLINA[1], M. MICELI[1], F. CAPANI[2], *L. R. GUELMAN[1];
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Abstract: It is well known that noise exposure can induce transient or permanent hearing loss. However, few data are available regarding its effects on extra-auditory structures, mainly within developing Central Nervous System. Previous studies of our laboratory showed that exposure of immature rats (7 and 15-days-old) to moderate noise during 2 hours, can induce hippocampal-related behavioral alterations that differ depending on the age of exposure. Moreover, rearing these animals in an enriched environment (EE) has shown to be an effective protective tool which almost fully prevented noise-induced behavioral changes.
Therefore, the aim of the present work was to test if noise exposure at different ages might generate hippocampal changes in an oxidative marker such as Trx-1. The potential prevention of these changes through the use of an enriched environment (EE) was also assessed.
Rats of 7 and 15 days were exposed during 2 h to white noise (95-97 dB) for one day. After weaning, groups of 3-4 rats were transferred to an enriched cage, consisting of toys, a wheel, tunnels and ramps, while other groups were placed in standard cages. After one week, levels of Trx-1, a member of the family of the antioxidants thioredoxins, were evaluated.
Results showed that Trx-1 levels of rats exposed at 7 days and reared in standard cages were increased, whereas no significant changes were found in rats reared in EE when compared with their respective control animals. In contrast, animals exposed at 15 days showed no significant differences in Trx-1 levels, neither in standard nor in enriched conditions.
These findings suggest that noise exposure at several developmental ages might differentially affect hippocampal oxidative status. The restoration of hippocampal Trx-1 levels in exposed animals reared in an EE might be correlated with a more reduced cellular milieu facilitated by EE rearing, suggesting that this strategy could be useful to aid animals to cope with an unfavorable condition.

Disclosures: **S.J. Molina:** None. **M. Miceli:** None. **F. Capani:** None. **L.R. Guelman:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 497.14/F25

Topic: A.07. Developmental Disorders

Support: ERC-2013-AdG 341116-PressBirth

Academy of Finland

Sigrid Juselius Foundation

Jane and Aatos Erkko Foundation

Title: Arginine vasopressin at nanomolar concentrations suppresses bumetanide-sensitive network events in the perinatal rat hippocampus

Authors: *A. SPOLJARIC, P. SEJA, E. RUUSUVUORI, I. HIIRONNIEMI, J. LINDFORS, M. SUMMANEN, J. VOIPIO, K. KAILA;
Univ. of Helsinki, Helsinki, Finland

Abstract: During birth, a number of homeostatic and pre-adaptive mechanisms are activated in mammals to facilitate the abrupt transition to extrauterine life. Human studies have shown that birth triggers a massive release of arginine vasopressin (AVP) into the blood. However, there is no information on whether the AVP surge also acts centrally on cortical structures. We performed in vitro electrophysiological experiments on rat hippocampal slices and intact hippocampi at the end of the fetal period (E20-21), within 2h after birth, and during postnatal days 0-2. During the whole perinatal period, exposure to exogenously applied AVP (5-10 nM) produced a strong suppression of CA3-driven bumetanide-sensitive network events, known as Giant Depolarizing Potentials (GDPs), which was prevented by the V1a receptor antagonist SR 49059 (20 nM). Intracellular recordings from CA3 pyramidal neurons revealed that, while lacking a direct effect on intrinsic properties, AVP induced a pronounced V1a receptor-dependent increase in the frequency of spontaneous IPSCs (sIPSCs). The increase in sIPSC frequency was seen in both the presence and absence of ionotropic glutamate receptor (iGluR) blockers, indicating direct activation of interneurons by AVP. Additionally, a pronounced desynchronization of cellular activity took place in the absence of iGluR blockers, readily explaining the suppression of GDPs. Thus, we suggest that AVP exerts neuroprotective actions and prevents pathophysiological plasticity by suppressing neuronal network activity during birth. This conclusion has several important consequences, especially in the light of data showing that AVP release during human birth is further enhanced under pathophysiological conditions such as birth asphyxia.

Disclosures: **A. Spoljaric:** None. **P. Seja:** None. **E. Ruusuvuori:** None. **I. Hiironniemi:** None. **J. Lindfors:** None. **M. Summanen:** None. **J. Voipio:** None. **K. Kaila:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 497.15/F26

Topic: A.07. Developmental Disorders

Support: support from Nutricia Advanced Medical Nutrition

Title: Neurobiological and functional benefits of a specific nutrient combination in phenylketonuria (PKU): proof of concept in the PKU mouse model

Authors: *V. M. BRUINENBERG[1], E. VAN DER GOOT[1], D. VAN VLIET[2], M. L. DE VRIES[1], D. S. COUNOTTE[3], M. KÜHN[3], F. J. VAN SPRONSEN[2], E. A. VAN DER ZEE[1]; [1]GELIFES, Univ. of Groningen, Groningen, Netherlands; [2]Children Hosp., Univ. Med. Ctr. Groningen, Groningen, Netherlands; [3]Nutricia Advanced Med. Nutr., Utrecht, Netherlands

Abstract: Phenylketonuria (PKU) is an inborn error of metabolism causing the enzyme that converts phenylalanine (Phe) to tyrosine to become non-functional. This in turn leads to a distinct rise in Phe concentrations in blood and brain. The current method to manage the condition is a strict and early introduced diet of protein restriction and specific amino acid supplementation restricting Phe intake. If untreated, PKU leads to severe mental retardation. However, even in treated PKU patients the disorder manifests itself mostly at the level of the brain with changes in neurotransmitter metabolism, white matter integrity, synaptic functioning, and oxidative stress. The purpose of the present study was to investigate the effects of a specific nutrient combination (SNC) containing precursors and cofactors for the synthesis of neuronal membrane on behavioral and neurobiological deficits in PKU mice (BTBR Pah^{enu2}). To this end, we examined the impact of SNC in groups of 24 mice each (12 males, 12 females) with dietary treatment starting on postnatal day 31. The following six groups were compared: wild-type (WT), WT with SNC (WT+SNC), PKU high Phe (6.4 g/kg food), PKU high Phe+SNC, PKU low Phe (2.0 g/kg), PKU low Phe +SNC. Groups without SNC were fed an isocaloric control. The mice were tested in two learning and memory paradigms (novel object recognition (NOR) and spatial object recognition test (SOR)), and a motor performance test (balance beam) at three time points during the dietary intervention (3, 6 and 9 months after the start of the intervention). Here we demonstrate that the diet containing SNC restored the ability of the PKU mice to learn the NOR, but SNC did not improve motor performance in PKU mice despite a significant lower performance compared to their WT litter mates. The SNC diet was nearly equally effective in PKU mice treated with high and low Phe in their diet. The analysis of brain material will shed light on the mode of action of the SNC diet. In conclusion, preliminary data show a beneficial effect of a SNC in a PKU mouse model.

Disclosures: **V.M. Bruinenberg:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Nutricia Research, Nutricia Advanced Medical Nutrition, Utrecht, The Netherlands. **E. van der Goot:** None. **D. van Vliet:** None. **M.L. de Vries:** None. **D.S. Counotte:** A. Employment/Salary (full or part-time): Nutricia Research, Nutricia Advanced Medical Nutrition, Utrecht, The Netherlands. **M. Kühn:** A. Employment/Salary (full or part-time): Nutricia Research, Nutricia Advanced Medical Nutrition, Utrecht, The Netherlands. **F.J. van Spronsen:** F. Consulting Fees (e.g., advisory boards); Nutricia Research, Nutricia Advanced Medical Nutrition, Utrecht, The Netherlands. **E.A. van der Zee:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Nutricia Research, Nutricia Advanced Medical Nutrition, Utrecht, The Netherlands.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 497.16/F27

Topic: A.07. Developmental Disorders

Support: Department of Neurology R&D funds

Title: Toward a therapeutic trial in Pelizaeus Merzbacher disease (PMD)

Authors: G. N. TURSKI[1], C. WESTMARK[1], *L. TURSKI[3], I. DUNCAN[2], C. IKONOMIDOU[1], C. A. TURSKI[1];
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Abstract: PMD is a rare dysmyelinating disorder of the CNS that is inherited in an X-linked recessive manner. The prevalence of the disease is about 1/100,000-200,000. The disease results from duplication of or a mutation in the proteolipid protein (*PLP1)* gene; proteolipid protein (PLP) is the major CNS myelin protein. The majority of boys with PMD have a duplication of *PLP1* (60-65%) while the remainders have a point mutation of the gene. Affected boys/men have significant neurologic impairment consisting of ataxia, rotary nystagmus, pyramidal signs, seizures, extrapyramidal movement disorders, psychomotor delay and intellectual disability. New observations on a canine animal model of PMD indicate that there is a gradual spontaneous differentiation of oligodendrocyte progenitor cells (OPCs) in the spinal cord but not brain

suggesting that drugs that could promote this earlier in the brain and spinal cord of PMD patients could have functional significance. Mei et al. (Nat Med 2014;20:954-960) identified a cluster of anti-muscarinic molecules that enhance oligodendrocyte differentiation and remyelination. One of these drugs is clemastine fumarate, an anti-histamine and anticholinergic medication that is licensed in the United States for treatment of allergic conditions. Clemastine has also been shown to promote *in vivo* remyelination in the lysolecithin induced demyelination mouse model. We tested the potential of clemastine fumarate to promote myelination in two rodent models of PMD, the myelin deficient (md) and the PLP transgenic rat. Pups were treated daily with clemastine (s.c.) doses of 10-30 mg/kg over 3 weeks (postnatal days 1-21). Neurologic phenotypes and myelination patterns in the brain, optic nerves and spinal cords were assessed using post mortem histological techniques. No change in neurological phenotype (whole body tremor, ataxia) was observed at the highest administered dose of clemastine, 30 mg/kg s.c. Postmortem staining with Luxol fast blue revealed islands of myelination in the brainstem of clemastine treated md and PLP rats. No evidence for improved myelination was found in the spinal cords or the optic nerves of treated rats compared to vehicle treated littermates who also developed the disease. Further studies are necessary to determine whether clemastine bears a therapeutic potential in this rare dysmyelinating CNS disorder.

Disclosures: **G.N. Turski:** None. **C. Westmark:** None. **L. Turski:** None. **I. Duncan:** None. **C. Ikonomidou:** None. **C.A. Turski:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

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Support: KAKENHI 23123519

KAKENHI 24659036

KAKENHI 26460073

KAKENHI 26860043

Mitsubishi Tanabe Pharma Co. Ltd

Title: Mice that lack the C-terminal region of Reelin exhibit behavioral abnormalities related to neuropsychiatric disorders

Authors: *K. SAKAI[1], H. SHOJI[2], T. KOHNO[1], T. MIYAKAWA[2,3], M. HATTORI[1];
[1]Biomed. Science, Grad. Sch. of Pharmaceut. Sci., Nagoya City Univ., Nagoya, Japan; [2]Div. of Systems Med. Science, Inst. for Comprehensive Med. Sci., Fujita Hlth. Univ., Toyoake, Japan; [3]Section of Behavior Patterns, Ctr. for Genet. Analysis of Behavior, Natl. Inst. for Physiological Sci., Okazaki, Japan

Abstract: Reelin is a large secreted glycoprotein that is required for normal brain formation and function. Reelin malfunction has been suggested to be assoiciated with the pathogenesis and deterioration of several neuropsychiatric disorders. Reelin protein is composed of the N-terminal domain, Reelin repeats, and the highly basic C-terminal region (CTR). Secreted Reelin binds to very low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2), and then induces phosphorylation of the intracellular protein Dab1, which leads to activation or modulation of further downstream signaling. The primary sequence of the CTR is conserved in most vertebrates and alternative splicing gives rise to a CTR-lacking isoform of Reelin. We previously revealed that the CTR of Reelin is necessary for efficient activation of its downstream signaling. We generated knock-in mice lacking the CTR of Reelin (ΔC-KI mice) and found that their brain structure is partially impaired. (Kohno et al. J. Neurosci. 35, 4776 (2015)) Here, we performed a comprehensive behavioral test battery on ΔC-KI mice, in order to evaluate the effects of partial loss-of-function of Reelin on brain functions. The ΔC-KI mice were hyperactive and exhibited reduced anxiety-like behavior in the open field test and the elevated plus maze test. Sociability of ΔC-KI mice was partially impaired in the home cage test, whereas social novelty preference was normal in Crawley's three-chamber social approach test. The working memory in ΔC-KI mice was impaired in the T-maze test. There was little difference in spatial reference memory, depression-like behavior, prepulse inhibition, or fear memory between ΔC-KI and wild-type mice. These results suggest that the CTR-dependent Reelin signaling is required for some specific brain functions. We think that the behavioral abnormalities of ΔC-KI mice recapitulate some aspects of neuropsychiatric disorders, such as schizophrenia, bipolar disorder, and autism spectrum disorder. The loss of Reelin CTR and/or reduced Reelin signaling could be the direct cause of some symptoms observed in these disorders. It was also suggested that ΔC-KI mice may serve as a unique tool for studying the mechanisms of neuropsychiatric disorders and their endophenotypes. We are now analyzing molecular mechanisms underlying behavioral abnormalities observed in ΔC-KI mice.

Disclosures: K. Sakai: None. **H. Shoji:** None. **T. Kohno:** None. **T. Miyakawa:** None. **M. Hattori:** None.

Poster

497. Preclinical Models for Neurodevelopmental Disorder Therapy

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Program#/Poster#: 497.18/F29

Topic: A.07. Developmental Disorders

Support: KAKENHI 23123519

KAKENHI 24659036

KAKENHHI 26460073

KAKENHHI 26860043

Mitsubishi Tanabe Pharma Co. Ltd.

Title: Does inhibition of Reelin processing ameliorate Alzheimer's disease?

Authors: *Y. YAMAKAGE, H. OGINO, T. KOHNO, M. HATTORI;
Dept. of Biomed. Science, Grad. Sch. of Pharmaceut. Sci., Nagoya City Univ., Nagoya, Aichi, Japan

Abstract: Alzheimer’s disease (AD) is the most common form of dementia and is characterized by loss of memory and cognitive dysfunction. The main cause of AD is the accumulation of amyloid beta peptide (Aβ) in the brain. Accumulated Aβ leads to neuronal cell death and synaptic failure.
Reelin, a large secreted glycoprotein composed of 3,461 amino acid residues, is required for normal synaptic functions in the adult brain. Reelin consists of the N-terminal region, eight Reelin repeats, and the C-terminal region. Reelin binds to its receptors via repeat 5 and 6, then the intracellular protein Dab1 is phosphorylated. Reelin is specifically cleaved within Reelin repeat 3 (N-t cleavage) and this cleavage virtually abolishes its biological activity. It was reported that the number and the size of Aβ plaques are decreased in Reelin transgenic mice (Pujadas et al., Nat. Comm. 5, 3443 (2014)) and that Aβ plaque pathology is worsened in Reelin conditional knock-out mice (Lane-Donovan et al., Sci. Signal. 8, ra67 (2015)). Moreover, the amount of full-length Reelin is decreased and the Reelin fragment generated by N-t cleavage is increased in human AD patients (Botella-López et al., Proc. Natl. Acad Sci. USA. 103, 5573 (2006)). These studies suggest that Reelin signaling is important to suppress AD pathology.
In this study, we hypothesized that the inhibition of N-t cleavage of Reelin would upregulate the biological activity of Reelin and might ameliorate the AD pathology. Our laboratory has clarified that A Disintegrin And Metalloproteinase with Thrombospondin motif 3 (ADAMTS-3) is a major protease that mediates N-t cleavage. We generated conditional knock-out (cKO) mice, ROSA26-CreERT2/ADAMTS-3-flox, in which administration of tamoxifen induces global loss

of ADAMTS-3. We crossed these mice with the knock-in mice in which pathogenic human amyloid precursor protein is expressed (Saito et al., Nat. Neurosci. 17, 661 (2014)). We are now investigating these mice to clarify whether the loss of ADAMTS-3 is beneficial for AD.

Disclosures: **Y. Yamakage:** None. **H. Ogino:** None. **T. Kohno:** None. **M. Hattori:** None.

Poster

498. Dopamine Signaling

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 498.01/F30

Topic: H.03. Schizophrenia

Support: NIH Grant R01MH090067

Title: Hippocampal and thalamic regulation of VTA dopamine neurons occurs via convergent inputs to the nucleus accumbens

Authors: ***S. M. PEREZ**, D. J. LODGE;
Pharmacol., UTHSCSA, San Antonio, TX

Abstract: Schizophrenia patients, and rodent models of the disease, display an enhanced hippocampal activity, which is thought to underlie augmented mesolimbic dopamine function associated with psychosis. Indeed, aberrant dopamine neuron activity can be restored in rodent models of schizophrenia by inactivating the ventral hippocampus (vHipp). In addition to vHipp hyperactivity, recent post-mortem studies have suggested that glutamatergic abnormalities are present in the nucleus accumbens (NAc) of individuals with schizophrenia. Specifically, an increase in vesicular glutamate transporter 2 (vGlut2) expression is observed. Increases in vGlut2 implies that individuals with schizophrenia receive aberrant glutamatergic input, specifically to the NAc. While hippocampal projections express some vGlut2, inputs from the thalamus are more likely to account for this alteration. Interestingly, anatomical studies suggest a convergent input from hippocampal and thalamic afferents to the NAc, which work in concert to regulate dopamine neuron activity. Here we examined the interaction between these pathways in the regulation of dopamine neuron activity in rodent models of schizophrenia. Electrophysiological experiments revealed that both hippocampal and thalamic activation induced a significant increase in population activity, and are dependent on each other. Similar to what was previously observed with vHipp inactivation in rodent models, TTX inactivation of the thalamus restored normal dopamine system function. Thalamus-induced increases in dopamine neuron activity are attributed to a direct projection to the nucleus accumbens. Furthermore, a subset of medium spiny neurons of the NAc receive convergent inputs from the vHipp and thalamus. We believe

that the vHipp is driving spontaneous membrane transitions (up/down states) that are required to enable the thalamic inputs to drive activity in the NAc. Further investigation of the role the thalamus plays in schizophrenia will provide a better understanding of the underlying pathophysiology of the disease, ultimately leading to novel sites of therapeutic intervention.

Disclosures: **S.M. Perez:** None. **D.J. Lodge:** None.

Poster

498. Dopamine Signaling

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 498.02/F31

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH grant MH057440-11

Title: Medial septum regulates spontaneous dopamine neuron activity in the ventral tegmental area and substantia nigra

Authors: ***D. M. BORTZ**, A. A. GRACE;
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Abstract: Dopamine (DA) neuron activity in the midbrain is associated with a wide array of essential behavioral constructs, including reward prediction and association, salience signaling, motivation, and fine motor control, and disruptions in this system are implicated in several psychiatric disorders. Although it is known that the ventral hippocampus potently regulates DA neuron spontaneous activity and that it receives a dense projection from the medial septum, little is known about how the septohippocampal pathway, a pathway known to impact learning and memory, affects the DA system. This was addressed by infusing NMDA (0.75 μg/0.2 μL) into the medial septum of anesthetized male Sprague-Dawley rats and recording dopamine neuron activity in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc). Septal stimulation produced a prolonged 72% increase in the number of spontaneously active DA cells in the VTA and a 96% increase in burst firing over vehicle infusions. Interestingly, septal stimulation produced the opposite response in the SNc, significantly decreasing spontaneous activity by 40% compared to vehicle. Moreover, ventral hippocampal inactivation by TTX reversed the effects of septal stimulation on DA neuron activity in both the VTA and SNc. In contrast to normal animals, septal stimulation in the MAM developmental disruption model of schizophrenia precipitated an opposite effect, producing a 53% decrease in dopamine neuron firing in the VTA and a 37% increase in the SNc. The number of spontaneously active DA

neurons is important for controlling the amplitude of the phasic dopamine response in the striatum, as only spontaneously active cells can burst fire in response to an incoming stimulus. Thus, these findings demonstrate that the septohippocampal pathway plays a key modulatory role in the regulation of meso-striatal DA transmission, impacting both the limbic VTA and the habit formation-related SNc DA neurons in a manner that is dependent upon the basal activity state of the system.

Disclosures: D.M. Bortz: None. **A.A. Grace:** None.

Poster

498. Dopamine Signaling

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 498.03/F32

Topic: B.05. Transporters

Support: NIH Grant R01AA019458

Title: Effects of ethanol, repeated high-dose methamphetamine & ceftriaxone treatments on dopamine, serotonin, glutamate, & glutamine tissue contents

Authors: Y. S. ALTHOBAITI[1], A. H. ALMALKI[2], S. C. DAS[2], F. S. ALSHEHRI[1], *Y. SARI[1];
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Abstract: Repeated exposure of high dose of methamphetamine (METH) is known to alter several neurotransmitters in certain brain regions. METH and ethanol are frequently consumed together by addicts with higher chance of reaching intoxication level. Little is known about the effects of METH and ethanol co-abuse on tissue contents of dopamine, serotonin (5-HT), glutamate, and glutamine in brain reward regions. In this study, Wistar rats were used as an animal model of METH and ethanol co-abuse to investigate their effects on tissue contents of dopamine/5-HT and glutamate/glutamine in nucleus accumbens (NAc) and prefrontal cortex (PFC). We further investigated the effects of ceftriaxone (CEF), a β-lactam antibiotic known to upregulate glutamate transporter subtype 1, on METH-induced alteration of these neurotransmitters. After seven days of either ethanol (6 g/kg) or water oral gavage pretreatment, rats received either saline or METH (10 mg/kg, i.p. every 2 hours x 4) followed by either saline or CEF (200 mg/kg, i.p, 3.) post-treatment. METH induced a significant depletion of dopamine and 5-HT in NAc and PFC. Interestingly, ethanol pretreatment potentiated the depletion of dopamine, but not 5-HT. Importantly, dopamine tissue contents were completely restored while

5-HT tissue contents were restored partially following CEF post-treatment in water group, but not in ethanol group, in NAc. Additionally, METH and ethanol caused differential effects in glutamate and glutamine tissue contents in PFC and NAc. These findings demonstrated for the first time the synergistic effect of METH and ethanol and the attenuating effects of CEF on these neurotransmitters in this co-abuse animal model.

Disclosures: Y.S. Althobaiti: None. **A.H. Almalki:** None. **S.C. Das:** None. **F.S. Alshehri:** None. **Y. Sari:** None.

Poster

498. Dopamine Signaling

Location: Halls B-H

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: MH002386

Title: Neuron-specific expression of the protein product of the Rapgef2 gene underlies activation of ERK by dopamine in the central nervous system

Authors: *Z. JIANG[1], A. C. EMERY[2], W. XU[2], T. MUSTAFA[2], C. R. GERFEN[3], M. V. EIDEN[1], L. E. EIDEN[2];
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Abstract: The Gs-coupled D1a receptor has long been known to signal through the cyclic AMP sensor protein kinase A (PKA) in the nervous system. We now identify two additional cyclic AMP sensors for D1a receptor signaling in neuroendocrine cells, the Rap guanine nucleotide exchange proteins Rapgef2 (NCS-Rapgef2) and Rapgef4 (Epac2). The use of three separate cyclic AMP sensors by D1 receptor activation allows parcellated activation of CREB (via PKA), ERK (via NCS-Rapgef2) and p38 (via Epac2) in the neuroendocrine cell line NS-1, as demonstrated by specific pharmacological inhibition of D1a/cAMP-dependent activation of CREB, ERK and p38 by the PKA-, NCS-Rapgef2-, and Epac2-selective inhibitors H89, SQ22,536, and ESI-09, and consistent with the previous demonstration of Gs-GPCR-coupled parcellated cAMP signaling in this cell line by the neuropeptide PACAP expressing the family B PAC1 receptor (Emery et al., Sci. Signaling 6(281), ra51, 2013). D1 signaling to ERK activation is known to be important for the dopamine-dependent actions of psychomotor stimulants in the nucleus accumbens. To determine if the NCS-Rapgef2 cAMP signaling pathway mediates D1-dependent activation of ERK in the central nervous system, an AAV vector expressing Cre recombinase under the control of the synapsin promoter was injected into the ventral striatum of

floxedRapgef2 mice. Four to five weeks later, these were treated with either the D1 agonist SKF 81297 (2 mg/kg, i.p.), or amphetamine (10 mg/kg, i.p.) and, 15 minutes later, euthanized, and perfusion-fixed for examination of ERK activation using an ERK Thr202/Tyr204 phosphospecific antibody. ERK activation following either psychomotor stimulant or D1 agonist treatment was robust in both shell and core of the nucleus accumbens on the side of the brain contralateral to AAV-syn-Cre-GPF injection but did not occur on the ipsilateral side, in cells which showed evidence of vector transduction (nuclear GPF staining) and corresponding loss of staining for the NCS-Rapgef2 protein. NCS-Rapgef2, is present in neuronal and neuroendocrine cell lines (NS-1, PC-12, SK-N-SH), but not non-neuronal cell lines (e.g. HEK293), and is correspondingly present in neuronal/neuroendocrine tissues (brain, pituitary, adrenal) but not in non-neuronal tissues (skeletal muscle, kidney, lung, spleen). Thus, we have identified a cyclic AMP sensor for the D1a and other Gs-GPCR-coupled receptors, which is expressed only in neurons within the CNS, and is required for dopamine-dependent activation of ERK in D1-dopaminoceptive medium spiny neurons of the nucleus accumbens core and shell.

Disclosures: **Z. Jiang:** None. **A.C. Emery:** None. **W. Xu:** None. **T. Mustafa:** None. **C.R. Gerfen:** None. **M.V. Eiden:** None. **L.E. Eiden:** None.

Poster

498. Dopamine Signaling

Location: Halls B-H

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Program#/Poster#: 498.05/F34

Topic: H.03. Schizophrenia

Support: National Ministry of Science (ANCyT)-PICT 2012-2024

Roemmers Laboratories

Fundación Florencio Fiorini

Fundación René Barón

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IBRO Travel Grant for SFN 2016

Title: Consequences of dopamine D2 receptor ablation on fast-spiking interneurons in mice

Authors: ***M. E. TOMASELLA**[1], C. MININNI[1], M. OGANDO[2], M. L. BECHELLI[1], M. DI GUILMI[3], B. ELGOYHEN[3], S. ZANUTTO[4], A. MARIN BURGIN[2], D. M. GELMAN[1];

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Abstract: Schizophrenia is a complex neurodevelopmental disease caused by both genetic and environmental factors. It is characterized by a heterogeneous collection of symptoms including altered perception, decreased motivation, and cognitive deficits. The dopaminergic hypothesis of the disease is the most enduring, as pharmacological treatment is based on antagonism of dopamine D2 receptors (DRD2). Strong evidence also demonstrate that Parvalbumin-expressing (PV), fast-spiking interneurons (FSI) have a central role in the pathophysiology of this disease, supporting the GABAergic hypothesis. Besides this, the glutamatergic hypothesis of schizophrenia has emerged based on reports showing that inhibition of NMDA (N-Methyl D-aspartate) receptors causes behavioral responses similar to the positive and cognitive symptoms observed in patients. Although great advances have been made in the last decades, and evidence supports each of the hypotheses, the molecular mechanisms leading to schizophrenia are still to be elucidated. Moreover, antipsychotics are the only treatment, with benefits just for positive symptoms, but limited results for negative or cognitive ones. As DRD2 is expressed in PV interneurons, the aim of the present work was to evaluate to what extent FSI inhibitory control over pyramidal neurons was modulated by DRD2. With this objective, we generated a conditional mutant mice line, by deletion of DRD2 specifically in PV+ interneurons. We first determined the mRNA expression level of many genes, as GAD 67, highly related to the pathophysiology of this disease. Our results showed a significant reduction in the mRNA of this and other relevant genes in the prefrontal cortex and hippocampus in conditional mutants compared to controls. Electrophysiological approach showed neuronal and network perturbations in conditional mutants, but not in control animals. We then followed a battery of behavioral tests to analyze the consequences of this deletion, focusing on locomotor activity, emotional and social behavior and cognitive function. Our results showed an alteration in total locomotor activity, impairments in cognitive capacity and deficits in social and emotional behavior in conditional mutants but not in control mice. In summary, our results show that deletion of DRD2 from FSI causes biochemical and physiological unbalances with functional consequences in behavior, suggesting that DRD2 exerts a fine tuning role in the development of a balanced activity in PV interneurons and, consequently, in the neuronal network.

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Poster

498. Dopamine Signaling

Location: Halls B-H

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Program#/Poster#: 498.06/F35

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Canadian Institutes of Health Research (CIHR)

Parkinson Society Canada

Title: Characterization of synaptic proteins expressed in dopaminergic axonal terminals

Authors: *C. DUCROT, M.-J. BOURQUE, G. FORTIN, G. CORREA, L.-E. TRUDEAU; Departments of pharmacology and neurosciences, GRSNC, Univ. De Montréal, Montreal, QC, Canada

Abstract: Dopamine (DA) neurons of the substantia nigra compacta (SNc) and ventral tegmental area (VTA) establish a complex axonal arborization comprising axon terminals that are either synaptic or non-synaptic in structure, as revealed by ultrastructural observations. No method other than electron microscopy has previously been used to examine the synaptic or non-synaptic nature of DAergic axonal varicosities. Our objective was to develop a rapid and efficient *in vitro* method for the quantification and analysis of synaptic and non-synaptic terminals established by mouse DA neurons. Considering previous works showing that subsets of DAergic neurons are able to package and release glutamate or GABA as some of their terminals, we took advantage of well-established postsynaptic markers of such synapses (respectively PSD95 and gephyrin), to characterize the axonal domain of these neurons. Primary DA neurons were prepared from the SNc or VTA of tyrosine hydroxylase (TH)-GFP transgenic mice and placed in co-culture with striatal neurons. Immunocytochemistry and confocal microscopy were then used to examine the colocalization of markers of the pre and postsynaptic compartments. Images were quantified using Image-J software. Our results show that the majority of axon terminals established by DA neurons contain the presynaptic markers synaptotagmin 1 (SYT1), SV2 and SNAP25. However, only a minority were found to be associated with the postsynaptic markers PSD95 or gephyrin. In comparison, mono-cultures of glutamatergic neurons from the cortex or GABAergic neurons from the striatum established a majority of terminals associated with a postsynaptic marker. These observations, revealing a fundamental difference between DA neurons and classical glutamate or GABA neurons, is compatible with previous ultrastructural results obtained from striatal brain sections and showing DA neurons to be predominantly non-synaptic. Finally, we also examined DA neurons from the olfactory bulb (OB), a neuronal population known to be structurally very different in their connectivity compared to VTA or SNc DA neurons. Interestingly, our preliminary results show that OB DA neurons are able to develop a majority of synaptic terminals, most of which are GABAergic. Our results validate the establishment of an

experimental strategy allowing quantification of the proportion of synaptic and non-synaptic contacts established by DA neurons, a technique that we will use to further explore the structure-function relationship of these axon terminals.

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Poster

498. Dopamine Signaling

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Topic: B.05. Transporters

Support: Danish Medical Research Council

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Lundbeck Fondation

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Title: Mice lacking PICK1 demonstrate increased dopamine levels and enhanced dopamine release in striatum associated with attenuated behavioral responses to cocaine

Authors: ***M. RICKHAG**[1], G. SØRENSEN[1], K. LOUISE JENSEN[1], D. DENCKER[2], W. ANTHONY OWENS[3], A. THOMSEN[1], T. RAHBEK-CLEMMENSEN[1], N. RIIS CHRISTENSEN[1], M. RATHJE[1], C. JIN[4], B. HOLST[4], A. FINK JENSEN[2], K. LINDEGAARD MADSEN[1], L. DAWS[3], U. GETHER[1];
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Abstract: Tuning dopaminergic signaling is critical for several physiological functions of the brain including motor behavior, reward and cognitive function. The presynaptic plasma membrane dopamine transporter (DAT) sequesters dopamine from the extracellular space, and thereby sustains physiological dopamine levels. DAT contains at its C-terminus, a PSD-95/Discs-large/ZO-1 (PDZ)-domain binding sequence, shown to interact with the scaffolding protein protein interacting with C-kinase 1 (PICK1). PICK1 regulates subcellular trafficking of

its binding partners but the explicit role of PICK1 for DAT function and dopamine homeostasis has remained elusive. Here, we present a detailed investigation of the dopaminergic system in mice lacking PICK1. Compared to wildtype (WT) mice, we found that DAT surface expression was unaltered in striatal terminals from PICK1 knock-out (KO) mice together with DAT localization to membrane microdomains. However, PICK1 KO mice were characterized by hyperlocomotion and attenuated locomotor response to cocaine although unaltered postsynaptic dopamine receptor activation. Intriguingly, PICK1 KO mice showed increased levels of tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis in striatum and elevated striatal dopamine content. Chronoamperometric recordings in striatum revealed elevated dopamine release in PICK1 KO mice supporting a hyperdopaminergic phenotype. PICK1 KO mice showed impaired behavioral sensitization to a cocaine-challenge and reduced chronic self-administration of cocaine suggesting long-term maladaptive plasticity changes. Lentiviral knock-down of PICK1 in midbrain dopaminergic cultures resulted in elevated TH expression and thus imply that PICK1 acts as negative regulator of dopamine synthesis. We infer that loss of PICK1 alters subcellular distribution of TH in striatum leading to elevated striatal dopamine content and development of a hyperdopaminergic phenotype.

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Poster

498. Dopamine Signaling

Location: Halls B-H

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: European Research Council starting grant 261286

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Novo Nordisk Fonden

Strategic Research Programme in Diabetes at Karolinska Institutet

Title: Dynamics of dopamine release in the median eminence studied by fast-scan cyclic voltammetry and optogenetic manipulation.

Authors: *A. S. STAGKOURAKIS, C. BROBERGER;
Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: Tuberoinfundibular dopamine (TIDA) neurons project to the median eminence (ME), where they release dopamine into the portal vasculature that drains into the adenohypophysis. At the target site, through its powerful inhibitory action on lactotroph cells, dopamine shapes the plasma profile of the hormone, prolactin. Prolactin mediates key functions in pregnancy, nursing and parental behaviours. Thus, the dynamics of TIDA neurons play a central role in the exhibition of behaviours critical for offspring survival. Recent work has revealed that TIDA neurons exhibit complex network interactions, including the ability to shift between phasic and tonic discharge. Yet, the relationship between the TIDA network electrophysiology and transmitter release is poorly understood.

In this study, the first in-depth analysis of optically and pharmacologically induced dopamine release using fast-scan cyclic voltammetry (FSCV) in the ME was performed. The dopamine transporter (DAT)-Cre mouse line was used to express Channelrhodopsin-2 in TIDA neurons. Brains from adult mice (>2 months old) were sectioned into 300 μm-thick coronal hypothalamic slices with intact ME. Dopamine was detected with high sensitivity (detection threshold; ≥5nM), with single 5msec light pulses evoking release of 28.20±9.59nM (n=6). Optical control of dopamine release exhibits minimal decay over time, with no difference on evoked dopamine levels upon repeated stimulations at five minute intervals. Prolonged 60sec tonic stimulation at 1Hz and 5msec pulse duration evoked dopamine release of 149.80±38.81nM (n=5), whereas constant 1sec light pulses led to release as high as 1μM (913.70±11.89nM; n=3). An inverted U-shaped frequency curve *vs* dopamine release suggests maximal release (53.67±33.18nM, n=3) at 10Hz matching the maximum spike fidelity *vs* firing frequency of TIDA neurons (10Hz, n=6). Phasic stimulation resulted in similar dopamine release as tonic stimulation in short term stimulation protocols (20sec), but long-term (5min) tonic stimulation resulted in diminished dopamine release as compared to sustained release in the phasic stimulation protocols. Lastly, application of the DAT blockers methylphenidate (50μM) and GBR-12783 (10μM), resulted in increased extracellular dopamine (41.50±14.82nM; n=4, and 623.00±202.50nM; n=5, respectively), suggesting the existence of endogenous dopamine release *in vitro*. Taken together, these results suggest that dopamine concentration can be manipulated optogenetically or pharmacologically at the TIDA neuron terminals in the ME, providing a read-out of the relationship between TIDA neuron electrophysiology and dopamine release.

Disclosures: A.S. Stagkourakis: None. **C. Broberger:** None.

Poster

498. Dopamine Signaling

Location: Halls B-H

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: P3E-UDG-2016.

Title: Extracellular levels of glutamic acid and dopamine in dorsal striatum in animals with perinatal asphyxia

Authors: *J. M. ORTEGA IBARRA, SR[1], A. MORALES-VILLAGRAN[2], S. J. LÓPEZ-PÉREZ[2];
[1]Univ. De Guadalajara, Zapopan, Mexico; [2]cellular molecular biology, Univ. of Guadalajara, Zapopan, Mexico

Abstract: During the perinatal period, the brain is particularly sensitive to hypoxia. Newborn rats that are exposed to hypoxia shows apoptosis, neuronal loss and high glutamic (Glu) extracellular levels in the brain. The striatum is a component of main entrance of the basal ganglia, receiving a strong glutamatergic innervation from all cortical regions, and dopaminergic innervation from substantia nigra pars compacta. Insults such perinatal hypoxia affect dopaminergic and glutamatergic developmental patterns connectivity. There are few neurochemical studies *in vivo* to study the hypoxia effects. To analyze the stimulated release of Glu and dopamine (DA) in dorsal striatum this study used a microdialysis method, to estimate the neurotransmitters availability in this brain region. We used 11 posnatal days (PD) Wistar rats, which were asphyxiated by 45 min in a small sealed chamber; when the animals reached 20 PD, were subjected to microdialysis procedure in the dorsal striatum. Samples were collected every 5 min at a flow of 2.5 µL/min under basal conditions for 30 minutes, then a solution containing high potassium concentration (100 mM, during 30 min) was used to stimulate the neurotransmitters release. After, the potassium concentration was returned to baseline for 45 minutes. The collected fractions were used to measure Glu by an electroquimioluminicent method designed in this laboratory, whereas DA was measure by HPLC with electrochemical detection. The results showed that hypoxia induces a low extracellular level of DA, but not of Glu. Under potassium stimulation, we do not found any difference in DA release, whereas in Glu, the hypoxia animals had higher release than controls. The basal low level of DA could be a consequence of neuronal loss observed in substantia nigra in a previous work, so it is possible that the dopaminergic terminals in the striatum are less too, even if we do not analyze these. It is possible that the enzymatic production of DA are more efficient by overexpression of tyrosine hydroxylase. About Glu results, it is possible that the fall of DA levels, that it is known that

regulate the Glu release in striatum, explain, at least in part, the rise in Glu extracellular level after stimulation. Support: P3E-UDG-2016.

Disclosures: J.M. Ortega Ibarra: None. **A. Morales-Villagran:** None. **S.J. López-Pérez:** None.

Poster

498. Dopamine Signaling

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH DA10900

NIH AA022449

Russian Science Foundation 4-50-00069

Title: Cross-hemispheric dopamine projections have functional significance

Authors: *M. E. FOX[1], M. A. MIKHAILOVA[2], C. E. BASS[3], P. TAKMAKOV[4], R. R. GAINETDINOV[2], E. A. BUDYGIN[5], R. WIGHTMAN[1];
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Abstract: Dopamine signaling occurs on a subsecond timescale, and its dysregulation is implicated in pathologies ranging from drug addiction to Parkinson's disease. Anatomical evidence suggests some dopamine neurons have cross-hemispheric projections, but the significance of these projections is unknown. Here, we show unprecedented interhemispheric communication in the midbrain dopamine system of awake and anesthetized rats. In the anesthetized preparation, optogenetic and electrical stimulation of dopamine cells elicited physiologically relevant dopamine release in the contralateral striatum. Contralateral release differed between dorsal and ventral striatum due to differential regulation by D2-like receptors. In the freely moving animal, simultaneous bilateral measurements revealed dopamine release synchronizes between hemispheres and intact, contralateral projections can release dopamine in the striatum of 6-hydroxydopamine lesioned rats. These experiments are the first to show cross-hemispheric synchronicity in dopamine signaling and support a functional role for contralateral

projections. In addition, the data reveal that psychostimulants such as amphetamine promote the coupling of dopamine transients between hemispheres.

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Poster

498. Dopamine Signaling

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Program#/Poster#: 498.11/F40

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: A critical role for CREB-regulated transcription coactivators (CRTC1 and CRTC3) in the regulation of the human tryptophan hydroxylase-2(TPH2) gene expression

Authors: Y. NAWA[1], H. KANEKO[1], M. TSUBONOYA[1], T. HIROI[1], R. TAKAHASHI[2], *H. MATSUI[3,1];
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Abstract: Dysfunction of the central serotonin (5-hydroxytryptamine, 5-HT) system has been implicated in the etiology of the wide range of neurodevelopmental disorders, including depression, anxiety, obsessive-compulsive disorder, and autism. As the rate-limiting enzyme for the synthesis of central 5-HT, tryptophan hydroxylase- 2(TPH2) plays an essential role in the regulation of 5-HT neurotransmission and is thus a promising therapeutic target for the treatment of neuropsychiatric disorders. Previously we reported the NRSF-mediated negative transcriptional regulation of the human TPH2 (hTPH2) gene (Gentile MT et. al, J. Neurochem., 123:963-70(2012)). However, the mechanism by which hTPH2 gene expression is activated remains unresolved. In the present study, we further characterized how the hTPH2 promoter activity is regulated by cAMP-mediated signaling pathways. A 2-kb region of the hTPH2 gene (-1850/+141) was cloned into pGL4-Basic and its 5'-untranslated region (+10/+121; a region containing repression elements including NRSE) was deleted to construct reporter plasmids. Promoter activities were assessed by transient transfections into immortalized rat serotonergic RN46A cells. Overexpression of CREB and PKA-alpha (cAMP-dependent protein kinase catalytic subunit alpha), either alone or in combination, only caused marginal effects. Overexpression of either CREB-regulated transcription coactivator CRTC1 or 3 with PKA-alpha and CREB remarkably increased the hTPH2 promoter activity. PKA-alpha/CREB/CRTC-mediated increase in the hTPH2 promoter activity was abolished when the inverted CRE motif

was mutated. Whereas Sp-6-Bzn-cAMP (a PKA activator) increased the hTPH2 promoter activity under combined overexpression of CRTC1 or 3 and CREB, 8-pCPT-2'-Me-cAMP (the specific EPAC (exchange protein directly activated by cAMP) activator) did not show any appreciable increase in the hTPH2 promoter activity, corroborating that PKA activation is required for enhancing effects of CRTCs. CRTC-mediated increase in the hTPH2 promoter activity was attenuated either by overexpression of R314A-CREB (defective for interaction with CRTCs) or R301L-CREB (defective for interaction with CRE) instead of wild-type CREB. In contrast, overexpression of S131A-CREB (defective for phosphorylation by PKA) increased the hTPH2 promoter activity comparable to wild-type CREB, suggesting that CREB phosphorylation itself is not necessarily essential. Collectively, these results indicate that CRTCs play a critical role for positive transcriptional regulation of the hTPH2 gene via cAMP-mediated signaling pathways and interaction with CRE-bound CREB.

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Poster

498. Dopamine Signaling

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CONACYT 152326

Title: CaMKII does not modulate D3 receptor effect on [^{3}H]-Glutamate release in the rat subtantia nigra.

Authors: *L. BRIONES[1], R. SÁNCHEZ ZAVALETA[2], D. ERLIJ[3], J. ACEVES[2], B. FLORÁN[2];
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Abstract: Dopamine D3 receptors (D3R) are highly expressed in substantia nigra pars reticulata (SNr) therein modulating GABA release in from striatal projections. In striato-nigral terminals CaMKII modulates negatively D3R. Additionally, SNr receives glutamatergic projections from subthalamic nucleus (STN) that modulate its output activity. It is well known the presence of mRNA and D3R protein in subthalamic neurons. The effect of D3R on glutamate release has not been well explored and the role of CaMKII in its activity remains unknown. Using high

potassium-induced [^{3}H]-glutamate release in slices of SNr, we have examined the effect of D3R on release and its possible modulation by CaMKII. PD 128,907 [100 nM] inhibited dose dependent [^{3}H]-glutamate release an effect mediated by D3R because co-perfusion with GR 103691 [10 nM] prevented it. D3R does not modulate [^{3}H]-glutamate induced release by adenylyl cyclase (AC) stimulation by forskolin [10 μM]. In order to test if CaMKII modulates D3R activity in subthalamonigral terminals, we tested KN62 [4 μM] a CaMKII inhibitor. KN62 did not modify D3R effect in glutamate release. Dopaminergic denervation with neurotoxin 6-OHDA injected directly in medial forebrain bundle increases the effect of PD 128,907 on D3R activation by inhibiting [^{3}H]-glutamate release by 60%. We concluded that the activation of D3R in subthalamo-nigral terminals modulate glutamate release without inhibition of AC and control of CaMKII.

Disclosures: L. Briones: None. **R. Sánchez Zavaleta:** None. **D. Erlij:** None. **J. Aceves:** None. **B. Florán:** None.

Poster

498. Dopamine Signaling

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 498.13/F42

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Determination of the concentrations of d-amphetamine, neurotransmitters and various metabolites in microdialysates taken from the brains of freely-moving rats

Authors: *D. GILL[1], R. KULKARNI[1], L. PINDER[1], H. ROWLEY[1], M. VAN DAM[2], D. MASCHER[2], H. MASCHER[2], D. HEAL[1], S. CHEETHAM[1];
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Abstract: In a previous study, we demonstrated that d-amphetamine-induced increases in striatal dopamine efflux measured by microdialysis in freely-moving rats correlated with plasma drug concentrations (Rowley et al, 2012, Neuropharmacology **63**:1064-74). In this investigation, we have taken this one step further by exploring the relationship between d-amphetamine-induced changes in the efflux of dopamine and its metabolites in the nucleus accumbens (ACB) and acetylcholine in the frontal cortex (FC) and the concentration of d-amphetamine in the same microdialysate sample. Two 2.0mm microdialysis probes were stereotaxically implanted into the ACB (AP +2.2mm, ML ±1.5mm, DV -8.0mm relative to bregma) and FC (AP +3.2mm, ML ±2.5mm, DV -4.0mm) of isoflurane-anaesthetised male, Sprague Dawley rats (~300-350g). After ≥16hr recovery, 20min microdialysate samples (1.2μl/min artificial CSF (aCSF) or aCSF+1.0μM neostigmine) were taken from freely-moving rats for 3hr after d-amphetamine dosing.

Dopamine, DOPAC and HVA were measured by Alexys hplc-ecd and acetylcholine by ALEXYS™ uhplc-ecd (Antec). d-Amphetamine (amphetamine-D5 internal standard) was measured in 2.0µl samples by hplc-MS/MS with ESI positive ionization (Shimadzu, AB Sciex API 5000). All results are reported as mean±SE, n=3-8. d-Amphetamine (0.5mg/kg sc) produced rapid increases in the efflux of dopamine in ACB with a peak at 40min of 53.8±9.7fmol/5µl (476% baseline, $p<0.001$). There were concomitant decreases in DOPAC and HVA with maximum reduction to 944±47fmol/5µl (41% baseline at 60min, $p<0.001$) and 779±29fmol/5µl (66% baseline at 80min, $p<0.001$), respectively. d-amphetamine concentrations in ACB microdialysates followed an identical pattern with a peak increase of 44.8±12.9ηg/ml at 40min. In the FC, d-amphetamine produced a more gradual increase of acetylcholine efflux with a peak at 60min of 462±54fmol/10µl (389% baseline, $p<0.001$). The time-course and magnitude of d-amphetamine concentrations in FC microdialysates were very similar to those observed in ACB with a peak of 32.5±5.9ηg/ml at 40min. d-Amphetamine concentrations were highly correlated with the magnitude of increases in ACB dopamine ($r^2=0.967$) and FC acetylcholine ($r^2=0.879$). These findings demonstrate that it is feasible to measure the concentration of d-amphetamine in intracerebral microdialysate samples as well as neurotransmitters and their metabolites. These experiments have shown that the actions of d-amphetamine on the efflux of dopamine in the ACB and acetylcholine in FC are highly correlated with the concentration of the drug in the extracellular fluid surrounding the sampling sites.

Disclosures: **D. Gill:** A. Employment/Salary (full or part-time): RENASCI LTD. **R. Kulkarni:** A. Employment/Salary (full or part-time): RENASCI LTD. **L. Pinder:** A. Employment/Salary (full or part-time): RENASCI LTD. **H. Rowley:** A. Employment/Salary (full or part-time): RENASCI LTD. **M. van Dam:** A. Employment/Salary (full or part-time): pharm-analyt. **D. Mascher:** A. Employment/Salary (full or part-time): pharm-analyt. **H. Mascher:** A. Employment/Salary (full or part-time): pharm-analyt. **D. Heal:** A. Employment/Salary (full or part-time): RENASCI LTD. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); RENASCI LTD. **S. Cheetham:** A. Employment/Salary (full or part-time): RENASCI LTD. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); RENASCI LTD.

Poster

498. Dopamine Signaling

Location: Halls B-H

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant DA019670

NIH Grant DA033611

NIH Grant AG043458

NIH Grant AG044848

Title: Investigating the effects of common dopamine D2 receptor gene polymorphisms on D2/3 striatal and midbrain dopamine receptor availability with [^{18}F]-Fallypride PET: C957T (rs6277) as a key determinant

Authors: *C. T. SMITH[1], J. W. BUCKHOLTZ[2,3], L. C. DANG[1], A. M. TETREAULT[1], S. F. PERKINS[1], J. J. CASTRELLON[1], R. L. COWAN[4,1], R. M. KESSLER[5], D. H. ZALD[1,4]; [1]Psychology, Vanderbilt Univ., Nashville, TN; [2]Psychology, Harvard Univ., Boston, MA; [3]Psychiatry, Massachusetts Gen. Hosp., Boston, MA; [4]Psychiatry, Vanderbilt Univ. Sch. of Med., Nashville, TN; [5]Radiology, Univ. of Alabama Birmingham Sch. of Med., Birmingham, AL

Abstract: Little work has focused on the impact of single nucleotide polymorphisms (SNPs) in the DRD2 gene on dopamine D2/3 receptor availability (assessed as binding potential, BPnd) beyond Taq1A SNP effects on striatal BPnd. Here, we used positron emission tomography (PET) with the D2/3 tracer ^{18}F-fallypride to assess the effect of 3 DRD2 SNPs (Taq1A, rs1800497; C957T, rs6277; -141C Ins/Del, rs1799732) on striatal and extrastriatal BPnd in 84 healthy subjects (ages 18-37, m=24.2±5.1; 53.6% female; 69% Caucasian). We focused our SNP analyses on an *a priori* striatal ROI but also conducted exploratory whole-brain analyses. All DRD2 alleles were present in expected ratios and did not violate Hardy-Weinberg equilibrium. In contrast to prior PET studies performed in smaller samples, we observed no significant effect of Taq1A status on BPnd. However, C957T T allele dosage (TT>CT>CC) was positively associated (pFDR<0.01) with striatal BPnd: Right Putamen, k(cluster size)=395, T=4.41 at MNI coordinates 26, 8, -4; d (Cohen's d effect size)=0.69, 98% confidence interval (CI): 0.59, 0.92; Left Putamen/Caudate, k=422, T=3.83 at -24, 4, -2; d=0.68, CI: 0.59, 0.82; when applying our *a priori* striatal small volume correction. Our whole-brain C957T analysis also identified a large midbrain/pons cluster at 2, -28,-26 where T allele dose was associated with higher BPnd (k=169, T=3.63; d=0.65, CI: 0.59, 0.78). We also observed a -141C Ins/Ins > Del Carriers BPnd effect in a similar midbrain/pons area (k=145, T=3.47 at 0, -26, -28; d=0.64, CI: 0.59, 0.76). Additionally, we constructed multi-locus genotype scores from the three SNPS, and investigated the impact of these scores on fallypride BPnd. The addition of Taq1A A2 allele dosage weakened (d=0.45, CI: 0.30, 0.58) while the addition of Ins/Ins allele status strengthened (d=0.75, CI: 0.53, 0.94) the initial C957T effect (d=0.65) on midbrain BPnd. The addition of Taq1A to the combined C957T + Ins/Ins score decreased the multi-locus effect on midbrain BPnd (d=0.45, CI: 0.22, 0.59), while the effect on striatal BPnd improved minimally (d=0.67 vs. 0.64).
Our results replicate previous work showing C957T T allele dosage is positively related to striatal BPnd and identify a novel effect in the midbrain, which is strengthened with the addition

of Ins/Ins genotype status. Taq1A alone or in combination with the other tested DRD2 SNPs was not associated with striatal BPnd, above our initial C957T effect. Given the strong linkage between the 3 SNPs we studied here, it is possible that C957T drove previously published Taq1A effects on BPnd. These findings demonstrate that DRD2 SNPs beyond Taq1A impact individual differences in D2/3 receptor availability.

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Poster

498. Dopamine Signaling

Location: Halls B-H

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Program#/Poster#: 498.15/F44

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: MEXT/JSPS KAKENHI Grant Number 25430029

MEXT/JSPS KAKENHI Grant Number 25115006

Title: Pre-synaptic dopamine release is regulated by post-synaptic activity to induce neuronal plasticity in *Drosophila*

Authors: *K. UENO, M. SAITOE;
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Abstract: Dopamine is an important neurotransmitter required for various higher order brain functions, including locomotor activity, sleep and memory formation. However, its release mechanisms and precise roles in these functions remain largely unknown. In *Drosophila*, long-term enhancement (LTE) of cholinergic transmission from the antennal lobes (ALs) to the mushroom bodies (MBs) is a cellular correlate of olfactory memory, and occurs upon simultaneous stimulation of the ALs and the ascending fibers of the ventral nerve code (AFV). Previous meeting, we have reported that AFV information is mediated by glutamate/NMDA receptors on MBs. On the contrary, activation of D1-type DA receptor on MBs is sufficient to induce LTE. We monitored the dopamine release by fluorescent exocytosis probe, synapto-pHluorin, and found that it occurs when MB neurons are simultaneously activated by acetylcholine and glutamate signaling, thus AL and AFV information. We also found that dopamine release is induced only onto MB neurons that have been coincidently activated. Although these findings suggest that dopamine release may require synaptic output from MB

neurons to activate DA neurons that loop back to the MBs, dopamine release is not disrupted when synaptic transmission from MB neurons is inhibited. Next we considered another possibility, releasing may be regulated by a retrograde message released from MB neurons. By pharmacological and genetic studies, we identified a potential messenger, which is released from activated MBs and retrogradely induces dopamine release. This MB-dopaminergic neurons-MB loop mechanism explains how dopamine functions to reinforce associative learning, and how its release is restricted to reinforce specific associations.

Disclosures: K. Ueno: None. **M. Saitoe:** None.

Poster

498. Dopamine Signaling

Location: Halls B-H

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CONACYT 152326

Title: Motor effects of nigral D4 receptors in normal and hemiparkinsonic rats.

Authors: *M. RODRÍGUEZ[1], E. ESCARTÍN-PÉREZ[2], S. LOYA-LÓPEZ[3], D. ERLIJ[4], B. FLORÁN[3];
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Abstract: The level of GABA in the sustantia nigra pars reticulata is decisive for the firing of nigral neurons and therefore for the motor control. GABA release is modulated by presynaptically located D1 and D4 dopamine receptors. The role of the D4 receptor and its participation in motor behavior has been poorly studied, particularly on experimental Parkinson. To understand this, we performed in vivo microdialysis experiments in sustantia nigra of normal and hemiparkinsonic rats; we measured dopamine, GABA and glutamate levels. In hemiparkinsonic rats we activated dopamine receptors with L-DOPA methyl ester (10 mg/kg i.p.) and the animals were monitored by a system of motor activity recording of with microdialysis.
In normal rats local blockade of D4 receptors with L-745870 produces selectively increase of GABA levels (129 ± 29%) and not of glutamate or dopamine. Motor behavior was also increased significantly at the same time of blockade. Systemic administration of the antagonist did not modify any parameter. In hemiparkinsonic rats L-dopa plus antagonist D4 receptor L-745870

increased levels of the three neurotransmitters and further the level of GABA selectively. L-dopa, by itself increases activity causing contralateral rotation, which was significantly higher by blocking the D4 receptor. These data highlight the role of GABA in motor control by D4 receptor at pallidus-nigral pathway. The effects observed in the hemiparkinsonic rats suggest the possible use of D4 blockers in the treatment of Parkinson's disease.

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Poster

499. Neurotrophins

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 499.01/F46

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NWO

ZonMW

Title: Loss of function of human-specific Brain-Derived Neurotrophic Factor exon VIII-containing transcripts associated with familial schizophrenia

Authors: ***S. T. MUNSHI**, A. AYO MARTIN, C. BOUWKAMP, N. GÜNHANLAR, G. VAN WOERDEN, Y. ELGERSMA, V. BONIFATI, F. M. S. DE VRIJ, S. A. KUSHNER; Erasmus MC, Rotterdam, Netherlands

Abstract: Despite its high heritability, the genetic basis of schizophrenia is only recently coming into focus. Large cohort genome-wide association studies have converged on a diverse group of common variants, however establishing cellular models to investigate their neurobiological influence has remained challenging. In contrast, rare high-penetrance mutations have been more difficult to identify, but offer a unique opportunity for performing detailed mechanistic studies of schizophrenia pathophysiology. In particular, with the advent of pluripotent stem cell technology, modeling rare highly-penetrant mutations in patient-specific neural cells has provided a novel way forward.
We performed linkage analysis and whole-genome exome sequencing within a family identified with high incidence of schizophrenia and a Mendelian pattern of disease inheritance. Pluripotent stem cell derived-neural lineages were differentiated to perform detailed molecular studies of leading candidates segregating amongst all affected family members.
In total, twenty candidate genes were identified. Among them was a frameshift insertion leading

to a premature stop in exon VIII of *Brain-Derived Neurotropic Factor* (*BDNF*). The regulation of *BDNF* transcription is remarkably diverse, with more than 17 known alternatively spliced isoforms. Interestingly, exon VIII contains a human-specific start codon that results in a larger pre-domain of the protein with as of yet undetermined functions. Depolarization of multiple different neural lineage cell types revealed that exon VIII-containing transcripts are highly activity-dependent in neural precursor cells (50x) and moderately in neurons and astrocytes (5-10x). We identified two novel transcripts that contain exon VIII: transcripts IV-VIII-IX and VI-VIII-IX. Interestingly, exon IV and VI contain many regulatory epigenetic landmarks for altering gene expression and have previously been pleiotropically associated with multiple psychiatric disorders. When overexpressed in various neuronal lineages, BDNF VIII-IX protein levels were at least ten-fold lower compared to that of BDNF IX, suggesting a shorter half-life of exon VIII-containing isoforms.

Disclosures: **S.T. Munshi:** None. **A. Ayo Martin:** None. **C. Bouwkamp:** None. **N. Günhanlar:** None. **G. van Woerden:** None. **Y. Elgersma:** None. **V. Bonifati:** None. **F.M.S. de Vrij:** None. **S.A. Kushner:** None.

Poster

499. Neurotrophins

Location: Halls B-H

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Program#/Poster#: 499.02/F47

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Estonian Research Council (institutional research funding IUT19-18 and Grant ETF8844)

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Estonian Academy of Sciences

European Union through the European Regional Development Fund (Project No. 2014-2020.4.01.15-0012)

Title: Identification of novel regulatory mechanisms responsible for TrkB signaling-dependent transcription of BDNF in cortical neurons.

Authors: *J. TUVIKENE, E.-E. ESVALD, A. SIRP, E. ORAV, P. PRUUNSILD, T. TIMMUSK;
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Abstract: Brain-derived neurotrophic factor (BDNF), acting through its receptor TrkB, regulates the survival and differentiation of various neurons in the developing nervous system, and promotes synaptic plasticity in the adult brain. Previously, it has been shown that in neurons BDNF gene is a subject to an extensive autoregulatory positive feedback loop, where TrkB signaling induces BDNF mRNA expression. However, little is known about the molecular mechanisms responsible for the phenomenon. Here, we have elucidated the mechanisms behind BDNF autoregulation using rat primary cortical neurons together with various methods, including AAV-mediated overexpression of dominant negative forms of different transcription factors, luciferase reporter assays, and chromatin immunoprecipitation. Our findings improve the understanding of the regulation of BDNF gene transcription in cortical neurons.

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Poster

499. Neurotrophins

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Joint Brazilian-Swedish Research Collaboration STINT-CAPES

Fredrik och Ingrid Thurings Stiftelse

Title: The role of BDNF-trkB signaling in cortical parvalbumin interneurons during cognitive and emotional processes

Authors: *N. G. GUYON[1], C. LOPES AGUIAR[3], Y. XUAN[1], R. ANDERSSON[2], A. FISAHN[2], K. MELETIS[1], M. CARLÉN[1];
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Abstract: Prefrontal cortex (PFC) interneurons have a vital role in modulating cortical output and plasticity. They were shown to be involved in the regulation of attention, decision-making and memory. Dysfunctions in inhibitory parvalbumin (PV) interneurons have been implicated in

the pathogenesis of depression, anxiety and other neuropsychiatric disorders. Changes in the expression of the brain-derived neurotrophic factor (BDNF) and its receptor tyrosine receptor kinase B (trkB) in PV interneurons have been associated with the pathophysiology of schizophrenia. Furthermore, truncated trkB isoforms, unable to mediate normal neurotrophic response, have an increased expression in schizophrenic patients. These changes in the expression of the wild type and truncaded trkB receptors are correlated with altered GABA inhibition and local network oscillatory synchronization. To directly investigate how trkB signaling in cortical PV interneurons contribute to oscillatory activities in prefrontal cortex and cognitive and emotional behavior we generated Adeno-associated viruses with Cre-dependent expression of a dominant negative trkB receptor (trkB.DN; a truncated receptor that binds to BDNF but does not trigger intracellular signaling cascades). PV-Cre mice injected with trkB.DN into the PFC display normal locomotion but show aggressiveness and disturbances in behaviors related to memory, fear and anxiety. *In vivo* recordings reveal that the behavioral phenotypes are associated with higher delta-gamma phase-amplitude coupling in the PFC. This furthers our understanding of the relationship between BDNF-trkB signaling in PFC PV interneurons, schizophrenia-relevant behavioral alterations and aberrant states of prefrontal hyperactivity.

Disclosures: **N.G. Guyon:** None. **C. Lopes Aguiar:** None. **Y. Xuan:** None. **R. Andersson:** None. **A. Fisahn:** None. **K. Meletis:** None. **M. Carlén:** None.

Poster

499. Neurotrophins

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSFC Grant 31320103906

NSFC Grant 31271197

Title: Dose dependent effects of overexpressed BDNF from BDNF-gene transcripts with short 3'UTR in hippocampal CA1 neurons on memory formation

Authors: M. WANG[1,2], Y. ZHUAN[1,2], D. LI[1,2], P. P. SANNA[3], *T. BEHNISCH[1,2];
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Abstract: The Brain-Derived Neurotrophic Factor (BDNF) gene with its multiple transcripts takes part in mechanisms of learning and memory, but might have also the potential to promote

epileptogenesis. Here we present data showing that the dose of overexpressed BDNF determines whether memory formation is improved or worsened. A localized transduction of hippocampal CA1 neurons with AAV9-BDNF-short 3'UTR enhanced the expression level of BDNF to about 200% within two weeks. This dose of overexpressed BDNF improved the memory recall of a passive avoidance task and object location. In addition, key kinases of synaptic plasticity related signaling pathways showed enhanced phosphorylation levels, even three weeks after BDNF overexpression. A doubling of the BDNF doses by transduction of a higher number of CA1 neurons caused an increase in the mouse's anxiety level and a decline in the passive avoidance memory performance. In addition, a large number of animals developed seizure-like saliences. Thus, expression of BDNF from the transcript under investigation has the potential to improve memory formation in wild type mouse strains when the expression level of BDNF is well controlled.

Disclosures: **M. Wang:** None. **Y. Zhuan:** None. **D. Li:** None. **P.P. Sanna:** None. **T. Behnisch:** None.

Poster

499. Neurotrophins

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Topic: B.01. Neurotransmitters and Signaling Molecules

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VA Medical Research

Title: Effects of chronic intermittent ethanol exposure or forced swim stress on expression of BDNF exon variants in medial prefrontal cortex and hippocampus in C57BL/6J mice

Authors: *M. SOLOMON[1], R. I. ANDERSON[2], W. C. GRIFFIN[2], H. C. BECKER[2]; [1]Psychiatry, Med. Univ. of SC, Charleston, SC; [2]Med. Univ. of South Carolina, Charleston, SC

Abstract: We have previously shown that repeated cycles of chronic intermittent ethanol (CIE) exposure reduced BDNF mRNA and protein in the medial prefrontal cortex (mPFC). Additional

work found that the reduction in mRNA (Bdnf exon 4) after CIE also extended to the hippocampus (HPC) and, further, that reductions occurred after acute forced swim stress (FSS) in both regions. Interestingly, targeting transcripts with the Bdnf exon 2 variant revealed reductions only in mPFC after acute forced swim stress (FSS), suggesting differential regulation of Bdnf exon variants. Therefore, the present study was designed to extend these earlier findings by measuring mRNA levels of 7 known Bdnf non-coding exon variants (1, 2, 3, 4, 6, 7 & 8) that are spliced to the same coding exon (9), and measuring levels of exon 9 mRNA in mouse mPFC and HPC after a history of either repeated CIE exposure or after a single acute FSS. Adult male C57BL/6J mice received 4 weeks of CIE or air exposure in inhalation chambers (16 hr/day x 4 days/wk). Three days following the final CIE (or air) exposure, mice were sacrificed. A separate cohort of mice was exposed to a single 10-min FSS exposure and sacrificed 30 min later. After dissection and isolation of mRNA, expression of all 8 Bdnf mRNA variants was determined by real time quantitative PCR (qRT-PCR) using cyclophilin as the reference gene. Results revealed a diverse pattern of Bdnf exon variant mRNA expression. For instance, both CIE and FSS significantly *reduced* Bdnf exon 3 expression in the HPC by CIE (50±3.9%) and FSS (53±4.6%) (both $p<0.05$) compared to the CTL mice, but there was no reduction in mPFC. Bdnf exon 7 expression in the mPFC was *increased* by CIE (133±17.0%) and FSS (152±17.1%) compared to CTL (both $p<0.05$), but there was no change in the HPC. CIE and FSS exposure *increased* Bdnf exon 8 in mPFC (86±28.7% CIE, 114±29.8% FSS; both $p<0.05$) and HPC (51±15.6% CIE, 42±6.1% FSS; both $p<0.05$). Conversely, both conditions *decreased* exon 4 in the mPFC (22±7.4% CIE, 40±9.5% FSS) and HPC (42±4.0% CIE, 35±3.9% FSS) compared to CTL mice (all $p<0.05$). The BDNF coding exon (9) was found to trend toward a reduction in only the mPFC after CIE (21±4.4%; $p<0.06$). These results suggest there is complex regulation of BDNF expression that is selective to the type of experience and the brain region being analyzed. Since Bdnf exon variants have different subcellular localization that may allow for localized modulation of BDNF expression, ongoing work includes measuring BDNF protein expression in subcellular fractions from the mPFC and HPC after CIE or FSS.

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Poster

499. Neurotrophins

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Title: Endocannabinoid-BDNF interactions at cortical excitatory synapses

Authors: ***M. L.-W. YEH**, R. SELVAM, E. S. LEVINE;
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Abstract: Endocannabinoids (eCBs) and neurotrophins, particularly brain-derived neurotrophic factor (BDNF), are potent neuromodulators that are highly expressed throughout the mammalian neocortex. Both eCBs and BDNF play critical roles in many behavioral and neurophysiological processes and are primary targets for the development of novel therapeutics, specifically in relation to depression, anxiety and schizophrenia. In mammalian neocortex, eCBs and BDNF bind primarily to the type 1 cannabinoid receptor (CB1R) and the high affinity trkB tyrosine kinase receptor, respectively. These receptors are expressed throughout the cortical mantle, with the highest levels of both trkB and CB1Rs found in layers II/III and V. Our laboratory and others have previously established that at glutamatergic synapses in these cortical layers, BDNF rapidly potentiates excitatory transmission by enhancing presynaptic glutamate release and modulating NMDA receptors. In contrast, we have recently shown that BDNF attenuates inhibitory transmission by inducing postsynaptic release of eCBs that act retrogradely to suppress GABA release from presynaptic terminals. It is not known whether BDNF also induces release of eCBs at excitatory synapses, which could have a mitigating or opposing effect on the direct effects of BDNF at these synapses. Here, we investigate the physiological interactions between eCBs and BDNF at excitatory synapses in layer V using acute slices from mouse somatosensory cortex. Consistent with previous results, we observed an increase in the frequency but not amplitude of spontaneous AMPA-mediated miniature excitatory postsynaptic currents (mEPSCs) after bath application of 0.2 nM BDNF. We also observed an increase in mEPSC frequency following exposure to the specific CB1R antagonist SR141716A, suggesting that tonic eCB signaling is present at these terminals. Bath perfusion of BDNF in the presence of SR141716A typically resulted in a greater increase in mEPSC frequency than BDNF alone, suggesting that BDNF-trkB signaling induced the release of eCBs at these excitatory synapses. Ongoing experiments are further exploring this issue with pharmacological manipulations targeting eCB synthesis, release, reuptake and degradation. We are also using immunohistochemistry to examine colocalization of trkB and CB1R at glutamatergic synapses. These observations bolster the growing evidence for cross-talk between eCB and BDNF signaling at cortical synapses.

Disclosures: **M.L. Yeh:** None. **R. Selvam:** None. **E.S. Levine:** None.

Poster

499. Neurotrophins

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 499.07/F52

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR 114965

CIHR 126109

NSERC RGPIN 402265-2011

Title: Netrin-1 promotes phosphorylation and membrane insertion of GluA1 in hippocampal neurons.

Authors: *I. V. BEAMISH[1], S. D. GLASGOW[2], S. LABRECQUE[4], A. MCKINNEY[3], P. DE KONINCK[4], P. SÉGUÉLA[2], E. S. RUTHAZER[2], T. E. KENNEDY[2];
[1]Neurol. and Neurosurg., Montreal Neurolog. Inst., Montréal, QC, Canada; [2]Neurol. & Neurosurg., [3]Pharmacol. & Therapeut., McGill Univ., Montréal, QC, Canada; [4]Inst. universitaire en santé mentale de Québec, Univ. Laval, Québec, QC, Canada

Abstract: Netrin-1 plays an important role in the establishment of neural circuits during development; however, the functional relevance of netrin-1 in the mature central nervous system remains unclear. Selective removal of the netrin receptor Deleted in Colorectal Cancer (DCC) from adult forebrain neurons results in significant impairments in long-term potentiation (LTP), a form of activity-dependent synaptic plasticity, suggesting a role for netrin-1 in learning and memory. Using electrophysiological recordings in adult hippocampal slices, our lab has recently found that netrin-1 potentiates synaptic responses via activation of DCC. LTP induction has been shown to trigger post-translational modifications of glutamate receptor subunits of the AMPA subtype, which regulate their subcellular trafficking and are correlated with alterations in dendritic spine volume. Here, we show that application of exogenous netrin-1 results in increased levels of CaMKII phosphorylation, as well as phosphorylation of critical serine residues on the GluA1 AMPAR subunit. We also show that application of netrin-1 increases fluorescence intensity of super ecliptic pHluorin-tagged GluA1 (SEP-GluA1) at synaptic sites. Additionally, we demonstrate that brief application of netrin-1 leads to long-term increases in the volume of thin type dendritic spines. Finally, depolarization of hippocampal neurons increases extracellular netrin-1, consistent with an activity-dependent release of netrin-1. These results point to a critical role for netrin-1 in the regulation of synaptic transmission and long-term plasticity via DCC-mediated modulation of the phosphorylation status of GluA1.

Disclosures: I.V. Beamish: None. **S.D. Glasgow:** None. **S. Labrecque:** None. **A. McKinney:** None. **P. De Koninck:** None. **P. Séguéla:** None. **E.S. Ruthazer:** None. **T.E. Kennedy:** None.

Poster

499. Neurotrophins

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Program#/Poster#: 499.08/F53

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR 114965

CIHR 126109

NSERC RGPIN 402265-2011

Title: Netrin-1 regulates synaptic transmission in CA1 pyramidal neurons of the adult mouse hippocampus.

Authors: *S. D. GLASGOW[1], I. V. BEAMISH[1], E. WONG[1], L. J. TRIGIANI[1], J. GIBON[1,3], E. HAMEL[1], A. MCKINNEY[2], P. SÉGUÉLA[1], E. S. RUTHAZER[1], T. E. KENNEDY[1]; [1]Dept. of Neurol. & Neurosurg., [2]Dept. of Pharmacol. & Therapeut., McGill Univ., Montreal, QC, Canada; [3]Univ. of British Columbia - Okanagan, Kelowna, BC, Canada

Abstract: Netrin-1 is a secreted protein that has been implicated in axon guidance during development; however little is known about its role in adulthood. Netrin-1 protein is readily detectable in the adult mouse hippocampus, suggesting potential roles in synaptic plasticity and learning and memory. Here, we report that brief application of netrin-1 increases thin-type dendritic spine volume, and leads to a significant increase in the frequency of miniature excitatory postsynaptic currents with no detected change in presynaptic function, indicating that netrin-1 can increase the number of excitatory synapses on CA1 pyramidal neurons. Moreover, transient application of netrin-1 induces a long-lasting potentiation of Schaffer collateral-evoked excitatory AMPA-mediated postsynaptic currents in CA1 pyramidal neurons in acute brain slices. We provide evidence that his potentiation is due to an increase in GluA1 AMPA receptor insertion and requires postsynaptic netrin receptor deleted-in-colorectal-cancer (DCC). DCC-mediated GluA1 insertion is dependent on PLC, intracellular calcium, PKC, and CaMKII, but not on NMDARs or mTOR. We also show that genetic deletion of netrin-1 from forebrain excitatory principal neurons results in attenuation of long-term potentiation (LTP), as well as deficits in spatial memory. Together, these findings identify a novel function for netrin-1 in the regulation of synaptic plasticity in the adult brain.

Disclosures: S.D. Glasgow: None. **I.V. Beamish:** None. **E. Wong:** None. **L.J. Trigiani:** None. **J. Gibon:** None. **E. Hamel:** None. **A. McKinney:** None. **P. Séguéla:** None. **E.S. Ruthazer:** None. **T.E. Kennedy:** None.

Poster

499. Neurotrophins

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 499.09/G1

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Eunice Kennedy Shriver National Institute of Child Health and Human Development Intramural Research Program

Title: Neuregulin targeting in central neurons

Authors: *D. VULLHORST, T. AHMED, I. KARAVANOVA, A. BUONANNO;
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Abstract: The Neuregulin/ErbB4 signaling pathway is an important modulator of GABAergic inhibitory interneurons, synaptic plasticity and neural network synchronization. Neuregulins are synthesized as membrane-bound proforms that require proteolytic processing by α- or β-secretases to shed their ectodomains and to initiate signaling. Until recently, it was generally assumed that all Neuregulins target to axons or axon terminals and to activate postsynaptic ErbB receptor tyrosine kinases in paracrine or juxtacrine fashion, based in large part on analyses of CRD (type III)-NRG1 function in the peripheral and central nervous systems. However, we recently demonstrated that NRG2, a major NRG isoform in the postnatal brain, accumulates as an unprocessed pro-form on cell bodies and proximal dendrites where it co-localizes with Kv2.1 potassium channels clusters at a subset of neuronal ER-PM junctions characterized by the presence of subsurface cisternae (SSCs). We also demonstrated that shedding of the signaling-competent NRG2 extracellular domain and subsequent autocrine initiation of ErbB4 receptor activation in GABAergic interneurons requires glutamate signaling via NMDA receptors. Therefore, these findings constitute a novel, non-axonal NRG signaling modality in central neurons. Here, we investigate the subcellular trafficking of other major NRG isoforms in cultured hippocampal neurons and in the brain, and begin to delineate the rules that govern their differential targeting to cell bodies vs. axons. Our preliminary data indicate that cell body accumulation, association with SSC-type ER-PM junctions and NMDA receptor-dependent ectodomain shedding are common properties of several NRG isoforms. These findings further support the notion that autocrine signaling in the cell body is a major mode of endogenous NRG/ErbB4 signaling that is potentially important to regulate excitability and/or synaptic transmission onto interneurons.

Disclosures: D. Vullhorst: None. **T. Ahmed:** None. **I. Karavanova:** None. **A. Buonanno:** None.

Poster

499. Neurotrophins

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 499.10/G2

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Upregulation of neurotrophins by S 47445, a novel positive allosteric modulator of AMPA receptors in aged rats and in humans

Authors: *S. BRETIN[1], F. CALABRESE[2], E. SAVINO[2], K. BERNARD[1], L. XUEREB[3], N. GUIGAL-STEPHAN[4], A. PONTISSO-MAHOUT[5], M. PUEYO[1], E. MOCAER[1], G. RACAGNI[2], M. RIVA[2];
[1]Inst. de Recherches Internationales Servier, Suresnes Cedex, France; [2]Dept. of Pharmacol. and Biomolecular Sci., Universita' degli Studi di Milano, Milano, Italy; [3]Pole of Expertise Methodology and Data Valorisation, Lab. Servier, Suresnes Cedex, France; [4]Expertise Biotech. and Biomarker Research, Inst. de Recherches Servier (I.d.R.S.), Lab. Servier, Croissy, France; [5]Expertise Biotech. and Biomarker Research, Inst. de Recherches Servier (I.d.R.S.), Inst. de Recherches Servier (I.d.R.S.), Croissy, France

Abstract: S 47445 is a potentiator of AMPA receptors that possesses both procognitive and antidepressant-like properties (L. Danober, ADPD 2015; S. Bretin ECNP, 2015). Here, the neurotrophic effects of S 47445 were assessed on both 18 months old aged rats (from 1 to 10mg/kg p.o.) and in healthy subjects (20 and 50mg). In rats, mRNA and proteins levels of Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin 3 (NT3) and Nerve Growth Factor (NGF) were investigated in prefrontal cortex (PFC), ventral (VH) and dorsal (DH) hippocampus after 2-week-treatment with S 47445. The effects of the age (vs 3 months/vehicle) and of the drug treatment (vs 18 months/vehicle) were analyzed using two-way ANOVA followed by Fisher's PLSD.The clinical study was an open, monocentric, phase I study in 24 healthy male and female subjects aged between 18 and 55 years. Subjects received orally for 10 days S 47445 at 20 mg (n=12) or 50 mg (n=12). Plasma samples were taken at 3 time points (D1 predose, D10 predose and D10 3h) and BDNF protein was measured with Mesoscale assays. Chronic effect (D10 predose - D1 predose) and acute changes (D10 3h -D10 predose) were analyzed separately using a Wilcoxon rank signed test.When compared to adult animals, aged rats show a significant decrease of total Bdnf mRNA levels in PFC ($p<0.05$), but not in VH or DH. Following S 47445 treatment, increases of Bdnf mRNA were observed both in PFC (1mg/kg; $p<0.05$) and DH (3 and 10mg/kg; $p<0.001$; $p<0.05$, respectively). Similarly, we found a significant age-dependent decrease of proBDNF and mature BDNF (mBDNF) in PFC synaptosomal fraction, which were normalized by S 47445 treatment (proBDNF: 1 and 10 mg/kg; $p<0.05$; mBDNF: all doses; $p<0.01$). Further, S 47445 was also able to upregulate mBDNF protein in DH (3 and 10 mg/kg, $p<0.01$; $p<0.05$). Last, S 47445 normalized the age-related defects of NT-3 protein (all doses;

p<0.01) in PFC and in DH (1mg/kg; p<0.01) and increased NGF protein in DH (1 mg/kg ; p<0.05).In healthy subjects, after a 10-day treatment, S 47445 significantly increased plasma BDNF protein at 20 and 50 mg, pooled results with both doses (median difference D10 predose - baseline = 40.6pg/mL; p<0.05). No difference on BDNF protein at D10 predose and 3hours after S 47445 treatment was observed suggesting a chronic effect rather than an acute one. In conclusion, S 47445 displayed neurotrophic effects in DH and PFC of aged rats and reversed the age-related deficits of mBDNF and NT3 proteins. S 47445 also presented a chronic effect on plasmatic BDNF levels in healthy subjects. On these bases, S 47445 has promising therapeutic potential in Alzheimer's disease and in Major Depressive Disorders.

Disclosures: **S. Bretin:** A. Employment/Salary (full or part-time): Laboratoire Servier. **F. Calabrese:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Laboratoire Servier. **E. Savino:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Laboratoire Servier. **K. Bernard:** A. Employment/Salary (full or part-time): Laboratoire Servier. **L. Xuereb:** A. Employment/Salary (full or part-time): Laboratoire Servier. **N. Guigal-Stephan:** A. Employment/Salary (full or part-time): Laboratoire Servier. **A. Pontisso-Mahout:** A. Employment/Salary (full or part-time): Laboratoire Servier. **M. Pueyo:** A. Employment/Salary (full or part-time): Laboratoire Servier. **E. Mocaer:** A. Employment/Salary (full or part-time): Laboratoire Servier. **G. Racagni:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Laboratoire Servier. **M. Riva:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Laboratoire Servier.

Poster

499. Neurotrophins

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 499.11/G3

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Conacyt Grant 239516 (J.V. S-V)

Title: Gas1 is expressed and released from neuronal primary cultures from mouse cerebellum and hippocampus

Authors: *E. BAUTISTA[1], N. ZARCO[1], P. VERGARA[1], R. AGUILAR-ROBLERO[2], J. V. SEGOVIA-VILA[1];
[1]CINVESTAV, Mexico City, Mexico; [2]Univ. Nacional Autónoma de México, Mexico City, Mexico

Abstract: Gas1 (Growth Arrest Specific1) is a pleiotropic protein that induces a variety of effects, including cell-cycle arrest, apoptosis and proliferation in different cell types. Employing immunohistochemical techniques we had previously observed that Gas1 is strongly expressed in GABAergic, glutamatergic, dopaminergic and cholinergic neurons in the hippocampus, thalamus, s*ubstantia nigra pars compacta* and in the ventral horn of the spinal cord, respectively. Interestingly, we detected a very faint expression of Gas1 in the granular cell layer (glutamatergic neurons) of the cerebellum. Based on these data, we decided to analyze the levels of Gas1 in hippocampus and in both the granular and the Purkinje layers of the cerebellum. We now report that Gas1 mRNA and protein are present in the Purkinje-molecular cell layer, and in the hippocampus but that it is not found in the granular layer of the cerebellum. Moreover, we also detected soluble Gas1 in the medium of neuron-enriched cell cultures obtained from hippocampus and from the Purkinje layer. Interestingly, and consistent with our previous data, we did not detect Gas1 in cultures of cerebellar granular cells. The present results indicate a differential pattern of expression of Gas1 in adult brain, not necessarily associated with neurotransmitter phenotype. Moreover, Gas1 is secreted from neurons suggesting it may act in both autocrine and paracrine manners in the brain.

Disclosures: E. Bautista: None. **N. Zarco:** None. **P. Vergara:** None. **R. Aguilar-Roblero:** None. **J.V. Segovia-Vila:** None.

Poster

499. Neurotrophins

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 499.12/G4

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: JSPS KAKENHI Grant 24500619

Title: Exercise in the presence of slight inhibition of GABAergic synapses up-regulate the synthesis of nerotrophin in the motor cortex

Authors: *H. MAEJIMA, K. TAKAHASHI, G. IKUTA;
Hokkaido Univ., Sapporo, Japan

Abstract: Neurotrophins positively modulate neuronal survival and plasticity in the brain. Physical exercise up-regulates the expression of neurotrophins including BDNF in the brain. $GABA_A$ is the most common neurotransmitter associated with IPSP in the brain. Some reports indicate that the inhibition of GABAergic synapses in the hippocampus enhances neural plasticity associated with the increased expression of BDNF, whereas the inhibition of GABAergic synapses removed the exercise-induced expression of nerotrophins in the motor cortex in our previous study. The objective of the present study was to investigate the effects of exercise and slighter inhibition of GABAergic synapses on the expression of neurotrophin in the mouse motor cortex. Female ICR mice at 30 weeks of age were assigned to four groups based on two factors (exercise and extremely slight inhibition of GABAergic synapses). The mice in the exercise groups performed moderate treadmill exercise (15 m/min, 60 min) every day. The slight inhibition of GABAergic synapses was induced by intraperitoneal administration of $GABA_A$ receptor antagonist (bicuculline, 0.25mg/kg). The intervention composed of exercise and bicuculline administration was continued for two weeks. After the intervention, the motor cortex was deprived for quantitative PCR analyses and immunoassays. The mRNA expression and protein level of BDNF were measured based on real time PCR and ELISA. All procedures were approved by the ethics committee for animal research of Hokkaido University. After the intervention for two weeks, bicuculline administration significantly increased the mRNA expression of BDNF, wheareas there was no effects of exercise on the expression. In the protein assay, neither bicuculline administration nor exercise modified the BDNF protein level, whereas exercise in the presence of bicuculline administration significantly increased the BDNF protein level. Taken together, the present study elucidated that the interaction between slight blockade of $GABA_A$ receptor and exercise up-regulated BDNF protein level in the motor cortex. One possibility is that slight blockade of $GABA_A$ receptor could positively contribute to the exercise-induced up-regulation of BDNF synthesis. It was suggested that exercise in the presence of regulated neuronal balance between EPSP and IPSP is crucial for the up-regulation or the down-regulation of neurotrophin syntheses in the motor cortex.

Disclosures: **H. Maejima:** None. **K. Takahashi:** None. **G. Ikuta:** None.

Poster

499. Neurotrophins

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 499.13/G5

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Inositol hexakisphosphate kinase-2 interacts with protein 4.1N in the brain.

Authors: *L. NAGPAL, C. FU, S. H. SNYDER;
Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Inositol hexakisphosphate kinases (IP6Ks) are responsible for the synthesis of the energy-rich Inositol pyrophosphates (PP-IPs) in mammals and regulate cellular functions including chemotaxis, telomere length, endocytic trafficking, exocytosis as well as apoptosis. Among such pyrophosphates, diphosphoinositol pentakisphosphate (IP7) and bis-diphosphoinositol tetrakisphosphate (IP8) have been extensively characterized. IP7 is produced in mammals by a family of inositol hexakisphosphate kinases, namely IP6K1, IP6K2 and IP6K3, which have distinct biological functions. Of these, Inositol hexakisphosphate kinase-2 (IP6K2), has been identified as a pro-apoptotic factor with the ability to sensitize cells to apoptosis. To identify the protein interactome of IP6K2, we subjected wild-type mouse brain tissue lysates to anti-IP6K2 antibody-mediated coimmuno-precipitation followed by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). This screen revealed robust binding of protein 4.1N with IP6K2. Protein 4.1N is the neuronal selective isoform of the erythrocyte membrane cytoskeleton protein, 4.1R, also known as erythrocyte membrane protein band 4.1 like protein isoform a or EBP41L1. Protein 4.1N is believed to confer stability and plasticity to the neuronal membrane via interactions with multiple binding partners, including the spectrin-actin-based cytoskeleton, integral membrane channels and receptors as well as membrane-associated guanylate kinases. To further assess the specificity of the observed interaction between IP6K2 and 4.1N, we separately immuno-precipitated IP6K1 and IP6K3 from brain tissue lysates of mice and failed to detect any association with the 4.1N protein. Thus, the interaction of 4.1N is evidently specific to the IP6K2 form of inositol hexakisphosphate kinases. We are presently characterizing functional implications of IP6K2-4.1N interactions.

Disclosures: **L. Nagpal:** None. **C. Fu:** None. **S.H. Snyder:** None.

Poster

499. Neurotrophins

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Program#/Poster#: 499.14/G6

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: RNF Grant 14-25-00072

Title: Chronically expressed BDNF and proBDNF differently regulate pre- and postsynaptic characteristics in primary neuron culture

Authors: *A. BORODINOVA, Y. SPIVAK, I. SMIRNOV, A. MALYSHEV, A. BOLSHAKOV;
Inst. of Higher Nervous Activity, Moscow, Russian Federation

Abstract: Brain-derived neurotrophic factor (BDNF) is a key regulator of synaptic structure and plasticity in developing as well as mature neurons. It is synthesized as a precursor protein (proBDNF) that is subjected to a proteolytic cleavage to mature molecule and a prodomain. Recent evidences suggest that proBDNF has its own biological role, frequently opposite to the BDNF functions. Neurotrophic balance is extremely important for normal brain functioning, especially during development, and abnormal proBDNF/BDNF ratio may play a role in pathogenesis of many neurological disorders, including autism and fragile X syndrome. In the current study we are testing the hypothesis that chronic changes in proBDNF and BDNF expression can differently regulate functional characteristics of the cortical neurons. For continuous neurotrophins overexpression we designed lentiviral constructs, carrying plasmid with coding sequence for either cleavage-resistant proBDNF (pCSC-proBDNF-CR) or proBDNF, which may be cleaved to a mature neurotrophin (pCSC-BDNF). Cultured cortical neurons were transduced with mentioned lentiviruses, and presynaptic effects of chronically expressed neurotrophins were analyzed by immunocytochemical staining of VGlut, which correlates to the quantity of excitatory synapses. It turned out that continuous (2 weeks) pCSC-BDNF overexpression increased the number of VGlut puncta in primary neuron culture (5,96±0,3 per unit of length) as compared to control pCSC (3,35±0,3), whereas cleavage-resistant BDNF precursor had no long-term effects on VGlut distribution (3,26±0,3) and, therefore, the density of excitatory synapses. Next, we estimated the changes in postsynaptic properties during continuous (1 and 2 weeks) neurotrophins overexpression. According to Western blotting data, pCSC-proBDNF-CR significantly decreased the level of the key postsynaptic protein PSD95 after 1 or 2 weeks overexpression (0,85±0,05 and 0,76±0,07, respectively) as compared to control pCSC. Interestingly, BDNF overexpression did not change the PSD95 protein levels over a period of one week (1,08±0,02) but decreased it after two weeks of incubation (0,74±0,17). Using patch-clamp recording we have analyzed spontaneous activity of neural cultures. Preliminary data suggests that pCSC-proBDNF-CR overexpression does not influence activity of cortical neurons, while pCSC-proBDNF overexpression significantly activates spontaneous activity. Our results suggest that proBDNF may be responsible for modulation of postsynaptic characteristics, whereas mature neurotrophin acts both pre- and postsynaptically potentiating synapses formation.

Disclosures: A. Borodinova: None. **Y. Spivak:** None. **I. Smirnov:** None. **A. Malyshev:** None. **A. Bolshakov:** None.

Poster

499. Neurotrophins

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 499.15/G7

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: New non-invasive way to rescue neurons in spinal cord injury via intranasal administration of nerve growth factor: pilot study

Authors: *A. DE BELLIS[1], L. ALOE[2];
[1]Neurosurg., Maria Rosaria Maglione Fndn. Onlus, Napoli, Italy; [2]Inst. of Cell Biol. and Neurobiology, CNR, Rome, Italy

Abstract: Nerve Growth Factor is known to play a critical protective role on a number of brain neurons in mammals, including humans. Nerve Growth Factor can be delivered to Central Nervous Sistem (CNS) via nasal route (Chen QX; 1998 Delivery of nerve growth factor to the brain via the olfactory pathway. J Alzheimers Dis 1:35-44) and has a protective action on forebrain, ippocampus (Catteno A, Towards non invasive nerve growth factor therapies for Alzheimer's disease. J Alzheimers Dis. 2008, 15: 255-283) in Alzheimer disease and Parkinson disease. However, its role in the Spinal Cord is not still unclear. Indeed, NGF does not cross the blood-brain barrier if injected subcutaneously or intravenously and another delivery method is therefore required. Hence the aim of this study was first to investigate whether purified NGF reaches spinal cord neurons and has any effect on the motor skills of rats with induced spinal cord injury (de Bellis A; Progress in Neuroscience 2012; Vol 1,N(1-4):83-90,2012;ISSN:2240-5127) and second to determine its effect on NGF concentrations and NGF-receptors in injured spinal cord neurons in adult rats when administered via the nasal cavity (Aloe L;Neural Regeneration Research 2014;Volume:9;Issue:10;Page:1025-1030). Adult male Sprague-Dawley rats with intact and injured spinal cord (SCI-induction T8-T10 by Yasargil's clip for 30') received daily intranasal nerve growth factor administration (via spray) in both nostrils for 1 day or for 3 consecutive weeks. We found an increased content of nerve growth factor (NGF) and enhanced expression of nerve growth factor receptor in the spinal cord 24 hours after a single intranasal administration of nerve growth factor in healthy rats, while daily treatment for 3 weeks in a model of spinal cord injury improved the deficits in locomotor behaviour and increased spinal content of both nerve growth factor and nerve growth factor receptors. These outcomes suggest that the intranasal nerve growth factor bypasses blood-brain barrier and protects damaged neurons in spinal cord injury. They also suggest the potential therapeutic role of intranasally delivered nerve growth factor for the neuroprotection of damaged spinal nerve cells (de Bellis A; Proceedings of 2016 Annual Meeting American Spinal Injury Association, Philadelphia, USA). A new non-invasive way to rescue neurons in spinal cord injury. The future perspective could be to use intranasal NGF in acute spinal cord injury for neuroprotection or as growth factor for stem

cell transplants in cronic spinal cord inury (de bellis A; Proceedings of First International Rita Levi Montalcini's Meeting: Neuroscience and Therapy, 2016 Bologna, Italy).

Disclosures: A. De Bellis: None. **L. Aloe:** None.

Poster

499. Neurotrophins

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Program#/Poster#: 499.16/G8

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: R21AG031467

R01AG041944

Title: Aging and an immune challenge interact to produce a prolonged, but transient reduction in hippocampal CA1 late-phase LTP and BDNF in a rodent model of delirium.

Authors: *N. TANAKA[1], G. P. CORTESE[2], R. M. BARRIENTOS[3], S. F. MAIER[3], S. L. PATTERSON[1];
[1]Biol., Temple Univ., Philadelphia, PA; [2]Neurol., Univ. of Wisconsin, Madison, WI;
[3]Psychology & Neurosci., Univ. of Colorado, Boulder, CO

Abstract: Previously high functioning aged individuals who experience an acute infection, injury or surgery are at increased risk for developing an abrupt cognitive decline (sometimes termed delirium) - a very common, rapidly developing, severe but often temporary impairment in attention and cognition. Even if these individuals recover from the delirium, they are at significantly greater risk of eventually developing dementia. Very little is known about the molecular mechanisms that render an aging brain more vulnerable to the deleterious effects of a secondary stressor. However, data from rodent models suggest that aging, but otherwise unimpaired, individuals react to an immune challenge with an exaggerated inflammatory response in the brain. Aging (24-month-old) Fischer Brown Norway (F344xBN) rats generally show no sign of significant physical or cognitive impairments. However, in response to signals triggered by a peripheral immune challenge (an intraperitoneal injection of *E. coli*), they produce higher levels of proinflammatory cytokines and produce them longer than their younger (3-month-old) counterparts. In the aging rats, hippocampal IL-1β levels are significantly elevated 4 hours after infection and remain so for 8 - 14 days. In contrast, in the young rats, IL-1β levels rise transiently but return to near basal levels within 24 hours (Barrientos et al., 2009). Interestingly, the prolonged elevation in IL-1β in aged infected animals is paralleled by

prolonged deficits in a long-term memory task (fear-conditioning). We have previously demonstrated that a memory-related form of long-lasting synaptic plasticity - theta burst-evoked late-phase long-term potentiation (L-LTP) - is impaired in hippocampal area CA1 of aged animals 4 days after injection of *E. coli*. Levels of mature brain-derived neurotrophic factor (mBDNF) - known to be important for long-term memory and L-LTP - are reduced in hippocampal synaptoneurosomes prepared from aged animals at the same time point. Here, we present the results of a time-course study to determine if the deficits in mBDNF and L-LTP persist in parallel with the elevations in IL-1β and the impairments in memory. Aged infected animals showed a reduction in mBDNF lasting as long as the alterations in IL-1β and the memory task (8 -14 days). Interestingly, the deficit in L-LTP took slightly longer to fully recover (sometime between 14 - 21 days) - suggesting a requirement for additional processes and molecular players. We are currently piloting the use of synaptoneurosomes for large-scale screening for immune-driven alterations in additional molecules (RNAs and proteins) necessary for the persistence of plasticity.

Disclosures: N. Tanaka: None. **G.P. Cortese:** None. **R.M. Barrientos:** None. **S.F. Maier:** None. **S.L. Patterson:** None.

Poster

499. Neurotrophins

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 499.17/G9

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: KAKENHI 25870256

Title: Monitoring and visualizing changes in the expression of BDNF gene using bioluminescence imaging

Authors: *M. FUKUCHI, H. MORI, A. TABUCHI, M. TSUDA;
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Abstract: Brain-derived neurotrophic factor (BDNF), a neurotrophin, plays a fundamental role in expressing a variety of neuronal functions in the brain. And also, it has been reported that alterations in the levels of BDNF expression are observed in the brain with neurodegenerative and psychiatric disorders including Alzheimer's disease, depression, and epilepsy. Therefore, monitoring and visualizing changes in the expression of BDNF gene would be useful for understanding changes in the levels of BDNF expression under physiological and pathological conditions in the brain. Recently, we generated a novel transgenic mouse strain to monitor

changes in BDNF expression using luciferase as an imaging probe. We successfully detected the activity-dependent induction of the expression of BDNF gene in primary culture of cortical cells prepared from the transgenic mice using a microscopic bioluminescence imaging. Furthermore, we could detect the bioluminescence signal from the head region of transgenic but not wild-type mice after administration of luciferin, a substrate for luciferase, by *in vivo* imaging. However, we also detected the signal from ear, limbs, and tail of the mice, probably due to the expression of BDNF gene in skin. These results indicate that alterations in the expression of BDNF gene can be continuously monitored and visualized using the transgenic mice both *in vitro* and *in vivo*, however, several improvements would be necessary for visualizing changes in the expression of BDNF gene in the brain.

Disclosures: **M. Fukuchi:** None. **H. Mori:** None. **A. Tabuchi:** None. **M. Tsuda:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.01/G10

Topic: B.02. Ligand-Gated Ion Channels

Support: NIDA grant DA014241

Title: Examining the role of CaMKIIα in α4β2* nicotinic receptor function

Authors: ***M. B. MILLER**[1], W. ZHOU[2], M. PICCIOTTO[2];
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Abstract: Tobacco use is the leading cause of preventable death worldwide, accounting for 6 million deaths each year. Nicotine, the primary psychoactive component in tobacco, exerts its effects on the nervous system through interactions with nicotinic acetylcholine receptors (nAChRs). The primary reinforcing properties of nicotine are mediated by high-affinity nAChRs containing α4 and β2 subunits (α4β2* nAChRs, where * represents the possibility of other subunits), which are both necessary and sufficient for nicotine reinforcement in animal models of addiction. Though the role of α4β2* nAChRs in nicotine-mediated physiology and behavior is well-established, the molecular mechanisms underlying receptor regulation and downstream signaling pathways are not completely understood. Recent work from our lab has identified several protein interactors of α4β2* nAChRs isolated from mouse and human brain. In this study, we aimed to elucidate the role of one of these interactors, CaMKIIα, in nicotinic receptor function and nicotine reinforcement. CaMKIIα is one of the most highly expressed kinases in the brain with well-established roles in synaptic plasticity, learning, and memory. We show that co-

expression of CaMKIIα-mRuby in HEK cells alters surface localization of exogenous α4β2 nAChRs. Using mass spectrophotometry, we also provide evidence that CaMKIIα phosphorylates the α4 nAChR subunit at a previously unidentified site in the intracellular M3/M4 loop, providing a potential mechanism for its effects on receptor localization. Lastly, although the roles of CaMKIIα in cortex and hippocampus have been studied extensively, little is known about its role in the mesolimbic dopamine system. Here, we show that CaMKIIα is expressed in both dopaminergic (DA) and non-DA neurons in the mouse VTA, suggesting possibly novel roles in DA signaling. Current studies are aimed at elucidating the role of the CaMKIIα-nAChR interaction in nicotine-mediated physiology and behavior.

Disclosures: **M.B. Miller:** None. **W. Zhou:** None. **M. Picciotto:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.02/G11

Topic: B.02. Ligand-Gated Ion Channels

Support: JSPS KAKENHI 26430025

Title: Nicotinic activation of protein kinase A modulates excitatory synapses in pyramidal neurons to regulate sound-evoked responses in the thalamocortical input layers of mouse primary auditory cortex

Authors: ***T. NAGAYAMA**, K. YAMASAKI, K. INAKUMA, H. D. KAWAI;
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Abstract: In the study investigating current source density (CSD) profiles in auditory cortex, systemic nicotine administration enhanced the characteristic frequency (CF) tone-evoked responses in layers 2/3 and 4 of primary auditory cortex (A1). This nicotinic enhancement continued about 40 minutes, and was dependent on α4β2* nAChRs activity (Kawai et al., 2011, J. Neurosci., 31, 14367-14377). In addition, this nicotinic activation was also dependent on extracellular signal-related kinase (ERK) activity (Intskirveli and Metherate, 2012, J. Neurophysiol., 107, 2782-2793). However, detailed mechanisms for the nicotinic enhancement need further investigation.
To investigate what molecules are involved in the mechanisms, we prepared synaptoneurosomes from the upper layers of auditory cortex that includes A1 and found that systemic nicotine administration increased the phosphorylation of ERK and Ser845 of GluA1, a subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs). We also found that

a protein kinase A (PKA) inhibitor, myr-PKI, inhibited the nicotine-induced ERK phosphorylation completely, while it significantly reduced the phosphorylation of Ser845 of GluA1, suggesting that the nicotine-induced increase of GluA1-Ser845 and ERK1/2 phosphorylation is mediated by PKA.
These biochemical data also suggest that PKA mediates nicotinic enhancement of sound-evoked responses. To test this possibility, we recorded tone-evoked local field potentials (LFPs). Nicotinic enhancement of tone-evoked LFP in layers 3/4 of A1 was associated with reduced onset latency, and increased 20-80 % slope and peak amplitude. As expected, myr-PKI local injection inhibited these nicotinic effects. To look for the site of nicotinic modulation, we recorded thalamocortical (TC) monosynaptic excitatory postsynaptic currents (EPSCs) and spontaneous EPSCs (sEPSCs) in auditory thalamocortical slices. We found that nicotine didn't modulate the TC synaptic transmission, but increased the amplitude of sEPSCs in neurons with apical dendrites without affecting inter-event interval (IEI) of sEPSCs. In the presence of myr-PKI, nicotine didn't induce the effect on sEPSCs in pyramidal neurons. These results suggest that the nicotinic enhancement of tone-evoked responses recruits intracortical excitatory synapses via PKA in pyramidal neurons.

Disclosures: T. Nagayama: None. **K. Yamasaki:** None. **K. Inakuma:** None. **H.D. Kawai:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.03/G12

Topic: B.02. Ligand-Gated Ion Channels

Title: Role of the nicotinic acetylcholine receptors in Alzheimer's Disease hyperactivity in prefrontal cortex

Authors: F. KOUKOULI, 75724[1], M. ROOY[3], *U. MASKOS[2];
[1]Inst. Pasteur, Paris cedex 15, France; [2]Inst. Pasteur, Paris Cedex 15, France; [3]ENS, Paris, France

Abstract: Alzheimer's Disease (AD) is the most common form of dementia. The condition predominantly affects the cerebral cortex and hippocampus and is characterized by the spread of amyloid plaques and neurofibrillary tangles (NFTs). But soluble amyloid-β (Aβ) oligomers have also been identified to accumulate in the brains of AD patients and correlate with cognitive dysfunction more than the extent of plaque deposition. Here, we developed an adeno-associated viral vector expressing the human mutated amyloid precursor protein (hAPP) sequence harboring three pathogenic mutations associated with early-onset familial AD. Intracranial injection of the

AAV into the prefrontal cortex (PFC) allowed the induction of AD-like deficits in adults, thereby modeling human pathology. The possibility to target a discrete brain region dissects regional vulnerability to AD lesions and potential spreading of the disease. AAV-hAPP expression caused accumulation of Aβ oligomers, microglial activation, astrocytosis and the gradual formation of amyloid plaques. Importantly, *in vivo* two-photon Ca^{2+} imaging at the site of Aβ peptide expression, in awake mice, revealed an increase in neuronal activity, a dysfunction characteristic of the pathology, already during the accumulation of soluble oligomers. Because an interaction of nicotinic acetylcholine receptors (nAChRs) and Aβ peptide has previously reported we used our AD model in mice knock-out for different nAChR subunits and investigated their role in AD hyperactivity. Our data suggest that specific expression of distinct nAChR subunits enables a diverse regulation of cortical activity in the AD brain and could provide a mechanistic basis for understanding the pathophysiology of the disorder.

Disclosures: **F. Koukouli:** None. **M. Rooy:** None. **U. Maskos:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.04/G13

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH R01 GM57481

Title: Areca nut alkaloids as selective nicotinic acetylcholine receptor partial agonists

Authors: ***N. HORENSTEIN**[1], R. PAPKE[2];
[1]Chem., Univ. Florida, Gainesville, FL; [2]Pharmacol. & Therapeut., Univ. of Florida, Gainesville, FL

Abstract: Arecoline, an active agent in areca nut (*Areca catechu*) and the betel quids prepared from it, has partial agonist activity toward nicotinic acetylcholine receptor (nAChR) subtypes that mediate nicotine addiction. The level of activity of arecoline for these receptors is roughly comparable to that of the smoking cessation drugs cytisine and varenicline, yet arecoline is far more selective for these β2* nAChR than are the current cessation drugs, which are also efficacious for peripheral β4* nAChR and the widely expressed α7-type nAChR. Application of areca nut infusion to α4β2 and α6β2 containing nAChR resulted in a weak partial agonist responses and a subsequent refractory period when the receptor became insensitive to ACh, as determined by two-electrode voltage-clamp (TEVC) electrophysiological measurements made in the *Xenopus* oocyte expression system. Control applications of areca extract to

untransfected *Xenopus* cells showed no effect. Arecoline, the major pyridine alkaloid from the areca nut, was also a weak partial agonist of the α4β2 nAChR, but did not show the same level of subsequent inhibition of the ACh response. The observation of both partial agonism and apparent desensitization by the areca extract provides the impetus for us to discover the active components responsible for this activity, with the hypothesis that such compounds will serve as the basis for development of new, more selective smoking cessation agents compared to varenicline and cytisine. We have surveyed the activities of cytisine, varenicline compared to arecoline, and the related compound, isoarecolone versus key nAChR. It is apparent that both arecoline and isoarecolone have a favorable and advantageous lack of agonist activity at α7, ganglionic, and muscle-type receptors while maintaining the ability to serve as partial agonists at α4β2 and α6β2β3α4β2 receptors, which are the targets for smoking cessation therapies. Further, in comparison of isoarecolone, arecoline, and ACh as muscarinic agonists at 10 μM, isoarecolone was noteworthy in its weak partial agonism, nearly 6-fold less than arecoline on cells expressing multiple muscarinic receptor subtypes and decreased to essentially zero on M1 receptors specifically. These observations suggest that there will be a useful chemical space in which compounds will be developed with enhanced selectivity for α4β2 and α6β2β3α4β2 nAChR with diminished activity at mAChR.

Disclosures: **N. Horenstein:** None. **R. Papke:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.05/G14

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01 DA035958

NIH Grant R21 DA026627

Title: Low dose alcohol modulates alpha6-containing nAChR heterologously expressed in human SH-EP1 cells

Authors: ***J. WU**[1], F. GAO[1], X. MA[1], D. CHEN[1], M. GAO[1], D. H. TAYLOR[2], B. J. EATON[1], P. WHITEAKER[1], S. C. STEFFENSEN[2];
[1]Barrow Neurolog Inst., Phoenix, AZ; [2]Brigham Young University, Provo, UT 84602, Provo, UT

Abstract: Alcohol use disorder (AUD) is a serious public health problem that affects 17 million Americans and results in tremendous social, legal and medical costs. Unlike other addictive

drugs (e.g., morphine, cocaine or nicotine) that have specific molecular targets, there is no clear target for ethanol (EtOH). For example, EtOH concentrations used in many investigations were too high (e.g., >50 mM) compared to the EtOH concentrations in human brain after alcohol drinking, and high dose EtOH likely produces non-specific modulations of a variety of receptors, ion channels, intracellular signaling cascades, and gene expression in the brain. Therefore, it is important to understand the molecular mechanisms underlying low dose (e.g., <10 mM) EtOH effects in the brain. Unfortunately, the molecular and cellular targets that mediate the sensitivity to low dose EtOH remain to be defined. nAChRs containing α6 subunits (α6*-nAChRs) show a highly restricted distribution in midbrain dopaminergic neurons that are associated with drug dependence and addiction. The fundamental goal of this study was to examine the effects of low dose EtOH on α6*-nAChR heterologously expressed in human SH-EP1 cells using patch-clamp recordings. Co-transfection of human nAChR α6(N-terminal)/α3(transmembrane domain) chimera and β2, β3 subunits into human SH-EP1 cells formed functional α6*-nAChRs. Patch-clamp whole-cell recordings demonstrated that nicotine (NIC) currents in SH-EP1 cells were highly sensitive to the α6 subunit selective antagonist α-conotoxin MII (1 μM NIC: MII IC_{50}= 10.3±1.2 nM, Hill coefficients=0.9±0.1, n=10). Nicotine concentration-response relationship curves showed that NIC EC_{50} values and Hill coefficients were 0.34±0.02 μM and 0.7±0.1 (n=10). Different concentrations of EtOH (from 0.01 to 50 mM) on 1 μM NIC-induced currents were tested, and we found that EtOH modulates NIC currents in an EtOH concentration-dependent manner. Low, but not high, doses of EtOH enhanced NIC currents, forming a bell-shaped EtOH concentration-efficient curve with a maximal potentiation effect (about 125%) between EtOH 0.1 and 0.5 mM. Ethanol (0.5 mM) potentiated NIC induced currents (1 nM to 30 μM) in a NIC concentration-dependent manner, characterized by a decrease in EtOH potentiation with increasing NIC concentrations with a maximal potentiation at 10 nM NIC (about 165%). In conclusion, our results demonstrate a functional α6*-nAChR transfected in human SH-EP1 cells that can be used as an excellent cell model to investigate α6*-nAChR function and pharmacology. Under patch-clamp recording condition, low dose EtOH modulates α6*-nAChR function as a positive allosteric modulator.

Disclosures: **J. Wu:** None. **F. Gao:** None. **X. Ma:** None. **D. Chen:** None. **M. Gao:** None. **D.H. Taylor:** None. **B.J. Eaton:** None. **P. Whiteaker:** None. **S.C. Steffensen:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.06/G15

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS064969

Title: A correlation between affinities simplifies receptor dose-response analysis

Authors: *A. AUERBACH;
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Abstract: Agonists activate receptors because they bind with a higher affinity to active vs. resting conformations. Muscle adult-type nicotinic receptors (AChRs) and GABAA receptors have two approximately-equivalent agonist binding sites. In these receptors E2/E0=(Kd/Jd)2, where E2 and E0 are the activation equilibrium constants with two and without any bound agonists and Kd/Jd is the resting/active equilibrium dissociation constant ratio. All four of these constants have been estimated independently in mouse AChRs. Experiments show that for a family of ACh-like agonists, the resting and active affinities are correlated: $J_d=K_d^m$, with m~2. Hence, the affinity ratio simplifies to $K_d^{(1-m)}$. This correlation indicates that the fraction of the total available agonist binding energy that is used up to form the low-affinity complex (1/m) is the same for all of the tested agonists (~50%). A binding-gating plot, which is a log-log plot of K_d (affinity) vs. E_2 (efficacy) for different agonists, shows a linear correlation in AChRs and $GABA_A$Rs. E_0 and m are receptor-specific and agonist-independent constants that shape the dose-response profile and can be estimated from the intercept and slope of this plot. In these receptors and at the level of equilibrium constants, it is possible to estimate affinity from efficacy and *vice versa*.

Disclosures: A. Auerbach: None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.07/G16

Topic: B.02. Ligand-Gated Ion Channels

Support: BBSRC PhD BB/J014400/1

Title: The opposing influences of nicotinic acetylcholine receptor subtypes in the medial prefrontal cortex

Authors: *M. H. SABEC[1], P. J. BANKS[1], G. R. I. BARKER[1], S. WONNACOTT[2], Z. I. BASHIR[1], E. C. WARBURTON[1];
[1]Physiol. and Pharamcology, Univ. of Bristol, Bristol, United Kingdom; [2]Biol. and Biochem., Univ. of Bath, Bath, United Kingdom

Abstract: Nicotinic acetylcholine receptors (nAChR) play an essential role in multiple medial prefrontal cortex (mPFC)-dependent cognitive functions, but the complex pattern of nicotinic modulations within the mPFC is still being revealed (Wallace et al, 2013). The influence exerted by nAChR activity in the mPFC is dependent on the receptor subtype, its subcellular loci, the cell-type, and to which cortical layer the cell belongs (Wallace et al, 2013, Poorthuis et al, 2013). It is therefore unsurprising that the precise influence of nAChR on specific long-range inputs required for associative functions of the mPFC, such as associative recognition, are not known. The presented work therefore investigates the involvement of two major subtypes of nAChR in associative recognition memory, and their regulation of transmission and plasticity at the hippocampal-mPFC pathway, an input critical to this form of memory (Warburton et al, 2010). A combination of *in vivo* and *in vitro* approaches were taken to investigate the role of nicotinic receptor modulation in the mPFC. Associative recognition memory was assessed in rats using an object-in-place (OiP) behavioural task which tests the capacity of the subject to combine object and spatial information. Intra-cortical cannulations targeted to the mPFC allowed pharmacological agents to be directly administered at acquisition, consolidation, or retrieval stages of the task. Neuromodulation by nAChR of the synaptic projection between hippocampus and mPFC was examined through slice electrophysiology: Whole-cell recordings were taken from layer V pyramidal neurons in the prelimbic region of rat mPFC slices whilst afferent hippocampal fibres were electrically stimulated.
The data presents a divergence in the roles of the two major subtypes of nAChRs, homomeric α7 and heteromeric α4β2 receptors. Subtype specific antagonism during the OiP task revealed that α7 nAChR are essential in the acquisition of associative memory but not its retrieval, whereas α4β2 nAChR were required for memory retrieval but not acquisition. The subtypes also yielded opposing influences on long-term plasticity of the hippocampal-mPFC pathway *in vitro*. A pattern of paired pre- and post-synaptic activity, which in control conditions induced a transient potentiation of hippocampal-evoked responses, produced long-term potentiation with α7 nAChR agonism, but was converted to long-term depression by α4β2 nAChR agonist application. Thus, the major nAChR subtypes have differing profiles of involvement in associative recognition memory, which may reflect their ability to induce LTP and LTD in the hippocampal-mPFC input that is essential for this behaviour.

Disclosures: **M.H. Sabec:** None. **P.J. Banks:** None. **G.R.I. Barker:** None. **S. Wonnacott:** None. **Z.I. Bashir:** None. **E.C. Warburton:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.08/G17

Topic: B.02. Ligand-Gated Ion Channels

Title: Effects of Phantasmidine on neuronal nicotinic ACh and serotonin type 3 receptors

Authors: *A. A. PANDYA[1], J. YAKEL[2];
[1]IAC / CRCD, UAF, Fairbanks, AK; [2]Lab. of Neurobio., Natl. Inst. of Envrn. Hlth. Sci., RTP, NC

Abstract: Phantasmidine is a condensed tetracyclic alkaloid obtained from the Ecuadorian poison frog *Epipedobates anthonyi*. We tested the functional effects of synthetic phantasmidine on various subtypes of neuronal nicotinic ACh receptors (nAChRs; α7, α4β2 and α3β2) and the serotonin 5-HT3 receptors (5-HT3ARs) that had been expressed in *Xenopus* oocytes using two-electrode voltage-clamp techniques. We found that phantasmidine is a full agonist for the α7 receptors (with EC50 values of 9.89 $\pm$ 0.90 μM), and a partial agonist for the α3β2 and α4β2 nAChRs (with an EC50 value of 19.0 $\pm$ 13 μM and 3.06 $\pm$ 0.19 nM, respectively). The relative peak amplitudes of responses induced by phantasmidine (compared to 1 mM ACh) is 1.08 $\pm$ 0.04, 0.32 + 0.04, and 0.25 $\pm$ 0.08 for α7, α4β2 and α3β2 nAChRs, respectively. For the 5-HT3ARs, phantasmidine has a lower potency than that seen with any of the nAChRs (an EC50 value of 29.1 $\pm$ 1.7 μM with a relative peak amplitude of 0.79 $\pm$ 0.05 compared to 10 μM mCPBG). The phantasmidine-induced currents for the α7 and α4β2 subtype of nAChRs, as well as the 5-HT3ARs, were completely blocked by methyllycaconitine (30 nM), dihydro-β-erythroidine (10 nM), and tropisetron (30 nM), respectively. This shows that the functional effects of phantasmidine are due to its binding to the agonist site on these receptors since competitive antagonists are able to inhibit its actions. In summary, phantasmidine is an agonist for α7, α4β2 and α3β2 nAChRs and the 5-HT3ARs with the potential for clinical applications.

Disclosures: **A.A. Pandya:** A. Employment/Salary (full or part-time): University of Alaska Fairbanks. **J. Yakel:** A. Employment/Salary (full or part-time): NIEHS / NIH.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.09/G18

Topic: B.02. Ligand-Gated Ion Channels

Support: DA035430

NS090903

Title: Smoking-cessation reagent block of nicotine-induced upregulation results from weak base "trapping" in α4β2 receptor-containing acidic vesicles.

Authors: *A. P. GOVIND[1], Y. VALLEJO[2], J. R. STOLZ[3], J.-Z. YAN[3], G. T. SWANSON[3], W. N. GREEN[1,4];
[1]Neurobio., Univ. of Chicago Dept. of Neurobio., Chicago, IL; [2]Natl. Inst. of Dent. and Craniofacial Res. at the Natl. Inst. of Hlth., Bethesda, MD; [3]Dept. of Pharmacol., Feinberg Sch. of Medicine, Northwestern Univ., Chicago, IL; [4]Marine Biol. Lab., Woods Hole, MA

Abstract: The nicotinic acetylcholine receptor (nAChR) ligand, varenicline (Chantix), is thought to promote smoking cessation as a partial agonist competing with nicotine for rapid nAChR activation on mesolimbic dopaminergic neurons. Here we tested whether smoking cessation reagents have additional longer-lasting actions on nAChR nicotine-induced upregulation. Varenicline and lobeline reduced nicotine-induced upregulation measured by α4β2-type nAChR (α4β2R) functional responses in HEK cells or ^{125}I-epibatidine binding to live cultured neurons and HEK cells. The reduction was not observed previously because it does not occur with membrane fragments, only live cells. Furthermore, the reduction in upregulation was not observed if the acidic pH in intracellular compartments was neutralized, suggesting that the actions of varenicline and lobeline were a consequence of weak base "trapping" within intracellular acidic compartments, as was described for nicotine in Xenopus oocytes (Jia et al. 2003. J Neurochem). The degree of trapping appears dependent on ligand pK_a and affinity for α4β2R binding sites. Nicotine and dihydro-beta-erythroid, which have lower pK_as and α4β2R affinities, were not significantly trapped while epibatidine, which has a higher pK_a and affinity, was trapped similar to varenicline and lobeline. α4β2Rs in small acidic vesicles were imaged using α4 subunits tagged with pH-sensitive pHluorin. The numbers of α4β2R-containing acidic vesicles and α4β2R levels in the vesicles increased in parallel to increases in ^{125}I-epibatidine binding. Our findings indicate that smoking cessation reagents alter nicotine-induced upregulation through a novel mechanism in which weak base trapping is enhanced by high-affinity α4β2Rs in acidic vesicles and by a nicotine-induced redistribution of α4β2Rs to acidic vesicles.

Disclosures: **A.P. Govind:** None. **Y. Vallejo:** None. **J.R. Stolz:** None. **J. Yan:** None. **G.T. Swanson:** None. **W.N. Green:** None.

Poster

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01DA006736

NIH Grant F30DA036964

NIH Grant T32GM007337

Title: Behavioral and receptor pharmacology of nicotinic agonist epibatidine in key brainstem pain modulatory nuclei

Authors: *F. J. JARECZEK, S. R. WHITE, C. M. SANDE, D. L. HAMMOND;
Dept. of Anesthesia, Univ. of Iowa, Iowa City, IA

Abstract: The adverse interaction between smoking and chronic pain has been known for decades. The prevalence of smoking among patients seeking pain treatment approaches 50%, and smoking exacerbates both the intensity and associated impairment of chronic pain. The growing body of literature documenting this association has led to the proposition of a positive feedback loop: individuals smoke in part to relieve their pain, but smoking actually exacerbates the pain. The mechanisms responsible for this adverse interaction remain poorly understood. While the analgesic efficacy of nicotinic acetylcholine receptor (nAChR) agonists in acute pain states is well established, their role in modulating chronic pain is less well characterized. The complete Freund's adjuvant (CFA) model of chronic pain was employed to test the hypothesis that persistent inflammatory nociception diminishes the antinociceptive efficacy of the selective α4β2 nAChR agonist epibatidine in key brainstem pain modulatory nuclei. Paw withdrawal latency to a noxious heat stimulus was used to evaluate the antihyperalgesic and antinociceptive effects of epibatidine microinjected in the rostral ventromedial medulla (RVM) or periaqueductal gray (PAG) of male rats. The effects of epibatidine were assessed both in naïve animals and in animals four days or two weeks after intraplantar CFA injection. Interestingly, pretreatment with an α4β2-selective antagonist demonstrated that the antinociceptive effects of epibatidine in naïve rats were mediated by α4β2 nAChRs in the RVM but not in the PAG. While the antinociceptive effects of epibatidine in the RVM were abolished after two weeks of inflammatory pain, the antihyperalgesic effects remained unchanged. Persistent inflammation did not alter the antinociceptive or antihyperalgesic effects of epibatidine in the PAG. Of note, radioligand binding studies revealed no differences in α4β2 nAChR number or binding affinity in either nucleus in CFA-treated rats. Additional behavioral experiments to probe pharmacological specificity of action demonstrated that, in a persistent pain state, epibatidine may no longer act at α4β2 nAChRs in the RVM. Rather, it may exert its antihyperalgesic effect through serotonergic signaling pathways. Taken together, these data suggest that plasticity in nicotinic signaling within the descending pain modulatory pathways may in part explain the adverse interaction between smoking and chronic pain observed in humans.

Disclosures: **F.J. Jareczek:** None. **S.R. White:** None. **C.M. Sande:** None. **D.L. Hammond:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.11/G20

Topic: B.02. Ligand-Gated Ion Channels

Support: HHMI BDSI

Lehigh University Mountaintop Project

NIDA 1R21DA033831

Title: The role of lynx2, a nicotinic receptor modulator, in extinguishing fear and anxiety

Authors: *K. R. ANDERSON, H. WANG, J. M. MIWA;
Biol. Sci., Lehigh Univ., Bethlehem, PA

Abstract: Anxiety disorders rank amongst the most disruptive diagnosed mental disorders and are simultaneously amongst the most prevalent. The anxiety response is a normal and adaptive reaction to a stressor, however disorders can develop when individuals cannot return to baseline once the stressor has resolved. There is currently an incomplete understanding of the biological underpinnings of anxiety and anxiety disorders. As such, current anxiety treatments only address the symptoms without treating the cause. To address the cause, it will be necessary to understand the molecular basis for anxiety. A clue to the molecular mechanism arises from the fact that many anxiety sufferers self-medicate by smoking, suggesting a role of nicotine's target system, the cholinergic system, in anxiety modulation. We are using a candidate gene approach focused on the role of a cholinergic modulator, *lynx2,* which is highly expressed in the basolateral aspect of the anxiety structure, the amygdala (BLA). *lynx2* proteins bind to and suppress cholinergic receptors (nAChRs). Consistent with its spatial expression, mice lacking *lynx2* (lynx2KO), demonstrate elevated anxiety levels across several assays (light-dark, open-field, etc.). We hypothesize that experience-dependent plasticity in the BLA plays a role in the return to baseline state, and that this is subject to cholinergic modulation. To address this we are pairing behavioral pharmacology assays and electrophysiology studies in the *lynx2* KO mice to study anxiety behaviors using light dark box and fear extinction assays. Understanding of how amygdalar output can be altered or restored by lynx and cholinergic pathways could help in the development of treatments for anxiety disorders.

Disclosures: K.R. Anderson: None. **H. Wang:** None. **J.M. Miwa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ophidion.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

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Topic: B.02. Ligand-Gated Ion Channels

Support: by NIH grant R21 DA026627 (P.W.)

Barrow Neurological Foundation Start-up Funds (P.W.)

Barrow Neurological Foundation Fellowship (M.M.W.)

Title: Occupation of the alpha4(+)/alpha4(-) subunit interface enhances function of the low sensitivity alpha4beta2-nicotinic acetylcholine receptor isoform by destabilization of receptor closed states.

Authors: *M. M. WELTZIN, A. A. GEORGE, R. J. LUKAS, P. WHITEAKER;
The Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Alpha4beta2-nicotinic acetylcholine receptors (nAChR) are the most prevalent central nervous system subtype. They exist as two isoforms with high or predominantly low sensitivity to nicotinic agonists [HS $(alpha4)_2(beta2)_3$- and LS $(alpha4)_3(beta2)_2$-nAChR, respectively]. Both isoforms contain two, high-affinity, canonical binding sites for agonists at alpha4(+)/beta2(-) subunit interfaces. The LS isoform has a third, non-canonical interface located at its alpha4(+)/alpha4(-) interface that binds acetylcholine (ACh) with lower affinity. The HS isoform displays ACh concentration response curves (CRC) that are monophasic. At low ACh concentrations, the LS isoform exhibits similar ACh potency and efficacy as the HS isoform. However, higher ACh concentrations that occupy the alpha4(+)/alpha4(-) interface of the LS isoform produce a second LS phase, four-times larger in maximal function. The resulting ACh CRC is biphasic, reflecting both smaller HS and larger LS responses. Single-channel patch-clamp recordings were used to define unitary responses to acetylcholine (ACh) at specific concentrations. We expressed either alpha4beta2-nAChR isoform by injecting *Xenopus* oocytes at alpha4:beta2 loose subunit cRNA ratios of 1:10 or 30:1 (biased to produce HS or LS receptors, respectively) and did recordings 3-6 days later. To evoke single-channel responses, we used 1.3 or 0.7 μM ACh (EC_{50} for the HS isoform or for HS responses of the LS isoform, respectively) or 30 μM ACh (EC_{50} for the LS isoform LS response). Parameters measured include unitary amplitude, channel conductance, open durations and closed dwell times. At low ACh concentrations, both isoforms show similar open and closed dwell time characteristics. However, consistent with previous studies, we verified that the HS isoform has one conductance state under these conditions, whereas the LS isoform exhibits two. Critically, at 30 μM ACh, sufficient to occupy the alpha4(+)/alpha4(-) binding site, the LS isoform retains similar open

dwell times and open amplitudes, but exhibits significantly reduced closed dwell times. Our results show for the first time that occupation of the alpha4(+)/alpha4(-) binding site drives increased macroscopic receptor function primarily via destabilization of closed states.

Disclosures: M.M. Weltzin: None. **A.A. George:** None. **R.J. Lukas:** None. **P. Whiteaker:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

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Topic: B.02. Ligand-Gated Ion Channels

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Title: Menthol enhances nicotine's actions on midbrain neurons

Authors: *B. J. HENDERSON, T. R. WALL, C. H. KIM, S. MCKINNEY, H. A. LESTER; Div. of Biol., Caltech, Pasadena, CA

Abstract: Understanding how menthol cigarette use leads to reduced cessation rates compared to non-menthol cigarettes requires identifying the neurons that are altered by nicotine, menthol, and endogenous acetylcholine. To address this, we used the conditioned place preference assay (CPP) to examine nicotine reward-related behavior and found that menthol, co-applied with nicotine, results in greater reward-related behavior than nicotine alone. Using cultured midbrain neurons and patch-clamp electrophysiology we found that menthol potentiates functional upregulation of nicotinic responses on dopamine (DA) and GABA neurons. We also found that menthol combined with nicotine enhances agonist-induced changes in DA neuron firing frequency when compared to nicotine alone. Finally, we observed that menthol combined with

nicotine upregulates α4* (but not α6*) nAChRs more than nicotine alone. These changes in midbrain neurons are likely a critical component in the increased reward-related behavior observed when menthol is combined with nicotine and may explain, in part, how menthol cigarette smokers exhibit reduced cessation rates. Support: National Institutes of Health (NIH) (K99DA040047 [BJH], DA017279, DA019375, DA033721, DA036061, DA037161, DA037743), and California Tobacco-related Disease Research Program (17RT0127).

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Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.14/G23

Topic: B.02. Ligand-Gated Ion Channels

Support: RO1 DA030929

The Goldfeder Family Undergraduate Research Grant

Title: Unorthodox acetylcholine binding sites formed by alpha 5 and beta 3 accessory subunits in alpha 4 beta 2* nicotinic acetylcholine receptors

Authors: A. JAIN[1], A. KURYATOV[2], *J. M. LINDSTROM[3];
[2]Neurosci., [3]Inst. Neurolog. Sci., [1]Univ. of Pennsylvania, Philadelphia, PA

Abstract: All nicotinic acetylcholine receptors (AChRs) evolved from homomeric AChRs in which all five subunits are involved in forming ACh binding sites at their interfaces. Heteromeric α4β2* AChRs typically have two ACh binding sites at the α4/β2 interfaces and a fifth accessory subunit surrounding the central cation channel. The β2 accessory subunits do not form ACh binding sites, but α4 accessory subunits do at the α4/α4 interface in $(\alpha4\beta2)_2\alpha4$ AChRs. α5 and β3 are closely related subunits that had previously been thought to act only as accessory subunits and not take part in forming ACh binding sites. AChRs containing α5 subunits are important in nicotine responses because knocking out α5 increases self-administration, and because the D398N variation in the α5 AChR gene is associated with nicotine dependence and lung cancer. In the brain, β3 has been found almost exclusively in association with α6, and specific AChRs containing β3 and α6 have been shown to promote dopamine release. Thus, ACh sites incorporating α5 and β3 have the potential to be important drug targets. In this study, the effect of agonists at various subunit interfaces was determined by blocking these interfaces with the

thioreactive agent, 2-((trimethylammonium)ethyl) methanethiosulfonate (MTSET) reacting with a cysteine substituted for a homologous residue in the minus side of α4. Through selective blockage of specific subunit interfaces, we show that $(\alpha4\beta2)_2\alpha5$ and $(\alpha4\beta2)_2\beta3$ AChRs can form functional ACh binding sites at α5/α4 and β3/α4 interfaces. MTSET, like MTSEA, can block all function of $(\alpha4\beta2^{L121C})_2\beta2$ AChRs in which alkylation of the minus side of β2 blocks both α4/β2 ACh binding sites. Alkylation of $(\alpha4^{T126C}\beta2)_2\alpha5$ and $(\alpha4^{T126C}\beta2)_2\beta3$ AChRs, where the minus side of α4 contains a cysteine, with MTSET showed around 45% block of response for $(\alpha4\beta2)_2\alpha5$ AChRs and around 30% block of response for $(\alpha4\beta2)_2\beta3$ AChRs. In addition, a biphasic ACh concentration/response curve showed that $(\alpha4\beta2)_2\alpha5$ AChRs have two high sensitivity ACh sites at the α4/β2 interfaces (EC_{50} = 1.38 ± 0.33 μM) and a low sensitivity ACh site at the α5/α4 interface (EC_{50} = 22.60 ± 5.75 μM). These unorthodox ACh binding sites using α5 or β3 in combination with α4 subunits are a target for specific site-selective agonist drugs. NS9283 is a site-selective agonist for the α4/α4 ACh binding site that potentiates activation of $(\alpha4\beta2)_2\alpha4$ AChRs. Similar drugs might be developed for other accessory ACh sites whose activation may trigger aversion to nicotine.

Disclosures: **A. Jain:** None. **A. Kuryatov:** None. **J.M. Lindstrom:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

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Program#/Poster#: 500.15/G24

Topic: B.02. Ligand-Gated Ion Channels

Support: Wings for Life Spinal Cord Research

VA Foundation for Healthy Youth

Title: Alpha 7 nicotinic receptor coupling to heterotrimeric G proteins modulates RhoA activation, cytoskeletal motility, and structural growth

Authors: ***J. KING**, N. KABBANI;
George Mason Univ., Fairfax, VA

Abstract: Nicotinic acetylcholine receptors (nAChRs) modulate synaptic growth and neuronal morphology. Ligand stimulation of the α7 nAChR has been shown to regulate large heterotrimeric GTP binding protein (G protein) signaling in various types of cells. Here, we demonstrate a role for α7 nAChR/G protein interaction in the activation of small monomeric RhoA GTPases leading to cytoskeletal changes in neurite growth. Treatment of cells with the α7

nAChR agonist choline or PNU-282987 was associated with an increase in RhoA activity and an inhibition in neurite growth. Specifically, choline treatment was found to attenuate the velocity of microtubule growth at the growth cone and decrease the rate of actin polymerization throughout the cell. The effects of α7 nAChR activation were abolished by expression of a dominant negative α7 nAChR deficient in G protein coupling. Proteomic analysis of immunoprecipitated α7 nAChR complexes from differentiating PC12 cells and synaptic fractions of the developing mouse hippocampus revealed the existence of Rho GTPase regulating guanine nucleotide exchange factors (GEFs) within the α7 nAChR interactome. The findings underscore the role of α7 nAChR signaling to the cytoskeleton during neurite growth through a direct modulation of G proteins.

Disclosures: J. King: None. **N. Kabbani:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.16/G25

Topic: B.02. Ligand-Gated Ion Channels

Title: Characterization of human alpha3beta2 nicotinic acetylcholine receptor kinetics reveals subtypes based on different subunit stoichiometries

Authors: D. C. JACKSON[1], M. HALL[2], *S. N. SUDWEEKS[1];
[1]Physiol. and Dev. Biol., [2]Neurosci., Brigham Young Univ., Provo, UT

Abstract: The α3β2 neuronal nicotinic acetylcholine receptor (nAChR) is a novel subtype whose behavior is largely uncharacterized. We have found the α3β2 (containing) nAChRs to be one of the predominant subtypes expressed at the mRNA level in rat hippocampal interneurons using single-cell RT-PCR. The α3β2 containing nAChRs are not widely expressed in other brain regions making this receptor subtype a plausible and specific target for cognitive therapies. Here we use voltage-clamp electrophysiology to characterize human α3β2 nAChRs expressed in *Xenopus laevis* oocytes. We injected the subunits at different ratios (1:5 and 5:1) and are able to distinguish receptor subtypes with differences in kinetic properties in response to acetylcholine. Receptors formed by the two ratios have statistically different EC50s and desensitization properties. These findings indicate that the human α3 and β2 nAChR subunits can form multiple functional receptor subtypes.

Disclosures: D.C. Jackson: None. **M. Hall:** None. **S.N. Sudweeks:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.17/G26

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH R01 GM57481

Title: Paradoxical interactions of alpha7 nAChR silent agonists and allosteric modulators; equilibration between desensitized states and persistent currents.

Authors: *R. L. PAPKE[1], K. MANTHER[1], G. A. THAKUR[2], M. DAMAJ[3], A. R. KULKARNI[2], D. BAGDAS[3], C. STOKES[1];
[1]Dept Pharmacol & Therapeut, Univ. Florida, Gainesville, FL; [2]Pharmaceut. Sci., Northeastern Univ., Boston, MA; [3]Pharmacol. and Toxicology, Virginia Commonwealth Univ., Richmond, VA

Abstract: Investigation of α7 nAChR as a therapeutic target has led to the discovery of promising new drug types, including ago-PAMs and silent agonists. Ago-PAMs are positive allosteric modulators (PAMs) that also produce direct allosteric activation (DAA). While DAA by the prototypical ago-PAM GAT107 is transient, a single GAT107 application followed by a drug washout results in a prolonged period of primed potentiation of acetylcholine responses lasting up to an hour or longer. Silent agonists have little or no efficacy for activating the α7 ion channel but induce desensitization states. Some of these desensitized states can be converted into channel-active states by PAMs. NS6740 is a silent agonist that induces prolonged nonconducting states that are insensitive to activation by acetylcholine but activatible by a PAM. A single application of 30 μM NS6740 leaves receptors in a PAM-sensitive state for over an hour. GAT107 and NS6740 therefore appear to stably induce different peri-activatible states, which can work in concert to produce large ion-channel responses, and with sequential applications these two drugs induce varying levels of activation that persist following washout. With 30 μM GAT107 application following 30 μM NS6740, there was a biphasic activation with an initial peak 60 ± 20-fold greater than the amplitude of a response to 60 μM ACh alone. Within 60 seconds, current decreased to a level about 20-fold higher than ACh controls. This late-phase current decayed with a time constant of about 10 minutes and was still detectable as a mecamylamine-sensitive baseline current after 1 hour. Drugs that are intermediate in their induction of desensitization or allosteric potentiation are also intermediate in their ability to induce persistent currents. Despite their seemingly opposite effects on channel activation, both GAT107 and NS6740 have been shown to be very effective analgetic agents in the same in vivo pain models. This suggests that the different peri-activational states induced by GAT107 and NS6740 have common signal transduction activity, which we hypothesize is due to similar

effects on the receptor's intracellular interactome that is independent of their opposing effects on channel activation.

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Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.18/G27

Topic: B.02. Ligand-Gated Ion Channels

Title: Calcium imaging with GCaMP6s reveals differential responses to nicotine in sex-segregated hippocampal cultures

Authors: *K. A. REES[1], A. H. MAHNKE[1,2], S. D. WEYAND[1], A. M. SHARP[1], U. H. WINZER-SERHAN[1,2];
[1]Neurosci. and Exptl. Therapeut., [2]Women's Hlth. in Neurosci. Program, Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Sex differences have been shown to occur at the cellular level, even before puberty and the onset of high rates of sex hormone production. We have previously shown that chronic neonatal nicotine treatment can have long term effects on hippocampal excitability in a sex-specific manner. Since calcium is an important regulator of cellular function, we studied intracellular calcium dynamics in hippocampal cells in response to acute and chronic nicotine using the genetically encoded calcium indicator, GCaMP6s. Sex-segregated primary hippocampal cultures were derived from postnatal day 1 mouse pups, and transduced with an AAV5-viral vector encoding GCaMP6s. After 14-15 days *in vitro*, cells were imaged using a confocal microscope. GCaMP6s fluorescent signal dynamics, indicative of changes in intracellular calcium levels, could be visualized in cell bodies, and into proximal and distal neuronal dendrites and spines, and glial processes and micro-domains. Male and female hippocampal cultures had similar neuron to glia ratios, gross neuronal morphology and equivalent AAV5 transduction rates of neurons and glia. However, male and female neurons showed different responses to acute nicotine administration. In the presence of bicuculline (10 μM), male and female neuronal somas showed differential responses to increasing doses of bath-applied nicotine (0.01 μM to 10 μM). Notably, at 0.1 μM nicotine, female neurons reached their peak calcium response ($p = 0.005$ compared to 0.01 μM nicotine) and exhibited more robust calcium signal amplitude compared to male neurons ($p = 0.042$) which reach their peak response at 10 μM nicotine ($p = 0.011$ compared to 0.01 μM nicotine). Furthermore, we examined the

effects of acute, bath-applied nicotine in cultures chronically exposed to 0.1 μM nicotine for 5 days. Data suggest that chronic nicotine treatment increased the overall calcium response amplitude to acute nicotine in neurons. Glia cells from male and female cultures changed firing patterns in response to acute nicotine, and chronic nicotine exposure increased their calcium dynamics to bath-applied acute nicotine. These data reveal novel sex differences in the response to acute and chronic nicotine administration in hippocampal cultures. The results demonstrate sex differences that occur at the cellular level and in the absence of sex hormones, and therefore, may be due to either differences in intrinsic sex programming or patterning *in utero* in response to hormone surges. The observed differential responses may explain sex-specific long-term changes in hippocampal excitability that are seen after chronic neonatal nicotine administration.

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Poster

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH U19 MH085193

Title: Rational design and development of ligands for the treatment of depression through partial agonism and desensitization of α4β2-nAChR: a collaborative project summary

Authors: *B. EATON[1], O. K. ONAJOLE[2,3], L. YU[3], H. ZHANG[3], J. LIU[3], G. P. VALLERINI[3], W. TUECKMANTEL[3], V. ALEXANDROV[4], K. CAVINO[4], S. K. CHELLAPPAN[4], A. GHAVAMI[4], T. HANANIA[4], L. A. DAVID[4], M. MANZANO[4], N. E. PATERSON[4], C. RUIZ[4], E. SABATH[4], M. TERRY[4], L. THIEDE[4], D. WANG[4], P. WHITEAKER[1], M. NYS[5], C. ULENS[5], A. MAZZOLARI[6], G. VISTOLI[6], A. B. SMIT[7], B. J. CALDARONE[8], D. BRUNNER[4], R. J. LUKAS[1], A. P. KOZIKOWSKI[3];
[1]Neurobio., Barrow Neurolog. Inst., Phoenix, AZ; [2]Dept. of Biological, Chem. and Physical Sci., Roosevelt Univ., Chicago, IL; [3]Dept. of Medicinal Chem. and Pharmacognosy, Univ. of Illinois at Chicago, Chicago, IL; [4]Psychogenics, Inc., Tarrytown, NY; [5]Lab. of Structural Neurobiology, Dept. of Cell. and Mol. Med., Katholieke Univ. Leuven, Leuven, Belgium; [6]Dept. di Scienze Farmaceutiche, Univ. degli Studi di Milano, Milano, Italy; [7]of Mol. & Cell. Neurobiology, Ctr. for Neurogenomics and Cognitive Res., VU Univ., Amsterdam, Netherlands; [8]Harvard NeuroDiscovery Ctr. and Dept. of Neurol., Brigham and Women's Hosp., Boston, MA

Abstract: Depression is among the most common and debilitating symptoms of psychiatric disorders and has thus been the subject of extensive study. Despite the successes so far, currently available drugs have a number of shortcomings including limited efficacy, a need for chronic administration to achieve efficacy, and undesirable side effects from nausea and loss of libido to suicidal ideation and mania. Most research has focused on improving currently available drugs, but gains have been modest. More desirable would be the development of new classes of antidepressants. We have done so via the National Cooperative Drug Discovery/Development Group (NCDDG) Program involving a multidisciplinary research partnership to discover superior agents to treat mental illness. Here we summarize the results of a five year collaboration involving three research groups pursuing the development of novel antidepressant drugs targeting α4β2 nicotinic acetylcholine receptors (nAChR). Using sazetidine-A as a template, medicinal chemists developed multiple series of novel ligands with modifications to the pyridine core, azetidine ring, and alkynyl side chain with the objective of designing α4β2-nAChR partial agonists of high specificity. These ligands were characterized *in vitro* across an array of nAChR subtypes, and those with the best functional profiles were advanced for behavioral testing, primarily utilizing the SmartCube® assay (Psychogenics, Inc.) and the Forced Swim Test. Isoxazole substitutions of the pyridine core of sazetidine-A produced a few high-affinity compounds, but generally reduced ligand activity and were abandoned. The azetidine ring was successfully replaced with more stable heterocyclic structures of five to seven atoms. Compared to analogs that retained the azetidine ring, compounds with expanded rings trended toward affinities decreased by three to ten-fold. This affinity decrease was impacted by substitutions for the alkynyl group in the distal side chain, with a cyclopropane spacer enabling affinities to be retained. Compounds were generally well tolerated by mice, and a number exhibited antidepressant-like qualities. Side effect and ADME profiles were favorable, but given the high potency of ligands in the *in vitro* assays, the doses required to achieve *in vivo* efficacy were surprisingly high. In light of the pharmacokinetics, we believe that *in vitro* vs *in vivo* discrepancies in potency are likely due to lower than expected permeability of, or efflux across, the blood brain barrier. Thanks to very high specificity, this higher than expected dose does not appear problematic, and several ligands are suitable candidates for further development.

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Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.20/G29

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH intramural research program

Title: Differential cAMP changes induced by α7 nicotinic acetylcholine receptor between hippocampal dentate granule cells and GABAergic interneurons

Authors: *Q. CHENG, J. L. YAKEL;
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Abstract: The activation of α7 nicotinic acetylcholine receptors (nAChRs) has been shown to improve hippocampal-dependent learning and memory. Recently, our studies showed that activation of α7 nAChRs potentiated glutamate release through calcium and subsequently cAMP rises. It is well known that α7 nAChRs are widespread among different cell types in the hippocampus. However it is unclear if α7 nAChRs mobilize differential signaling mechanisms among distinct neuronal populations. To address this question, we compared α7 nAChR agonist-induced current responses, calcium and cAMP changes between granule cells and GABAergic neurons in hippocampal dentate region. We cultured hippocampal slices from POMC-cre (dentate granule cell-specific cre line) and GAD2-cre (GABAergic interneuron-specific cre line) transgenic mice, and infected with AAV virus containing either floxed EPAC-H150 (cAMP sensor) or GCaMP6s (calcium sensor). Consistent with previous studies, application of the α7 nAChR-selective agonist choline (4 mM; in the presence of the α7 nAChR positive allosteric modulator PNU-120596, 5 μM) evoked larger currents (618±87 pA) in GABAergic interneurons than that of dentate granule cells (108±24 pA). This suggested that GABAergic interneurons have higher α7 nAChR density than granule cells. Next, we found that activation of α7 nAChRs induced a significant increase in intracellular cAMP levels in the somas of granule cells, but not of GABAergic interneurons. The increase of cAMP in granule cells was blocked by MLA, suggesting the requirement of α7 nAChR activation; and by NB001 (a selective AC1 inhibitor), suggesting the involvement of AC1. We hypothesize that the lack of cAMP responses is due to the expression of a large number of calcium-regulating proteins in GABAergic interneurons. Indeed, GCaMP6 imaging revealed robust and prominent calcium rises induced by choline and PNU-120596 application in the soma and processes of most granule cells; while the change of intracellular calcium of GABAergic neurons was rather small and slow in the soma because of a higher endogenous Ca^{2+}-buffering capacity. Increasing external calcium concentration to 10 mM enhanced calcium responses to activation of α7 nAChRs in GABAergic neurons, and conferred them the ability to produce a cAMP rise. This suggests that the restriction of the Ca^{2+} transient

could limit α7 nAChR's action in GABAergic neurons, at least concerning intracellular signaling cascades. Our findings also provide better understanding of the complex molecular mechanisms of the positive cognitive effects of α7 nAChR agonists in hippocampal circuits.

Disclosures: Q. Cheng: None. **J.L. Yakel:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.21/G30

Topic: B.02. Ligand-Gated Ion Channels

Support: Innovation Fund Denmark (Cognito)

Lundbeck Foundation

Title: The alpha7 nicotinic acetylcholine receptor partial agonists NS6740 and GTS-21 reduce LPS-induced TNF-alpha release from primary cultures of human microglia cells

Authors: *J. D. MIKKELSEN[1,2], M. H. SØRENSEN[1], L. H. PINBORG[1], H. H. HANSEN[1]; [1]Univ. Copenhagen - Rigshospitalet, Copenhagen, Denmark; [2]Bionomics Ltd, Adelaide, Australia

Abstract: The penta-homomeric alpha7 nicotinic acetylcholine receptor (alpha7 nAChR) is a potential drug target for alleviating cognitive symptoms. Polymorphisms in the alpha7 nAChR gene (*CHRNA7*) and the duplicated alpha7 nAChR gene (*CHRFAM7A*), both show genetic linkage to schizophrenia. *CHRFAM7A* is truncated and not coding the ligand binding domain, and is a gene that in contrast to *CHRNA7*, is unique to humans. Due to the lack of the ligand-binding domain, co-transfection of the *CHRFAM7A* gene product (dup-alpha7) with the primal alpha7 nAChR subunit suppresses ACh-induced alpha7 nAChR currents in oocytes. The dominant-negative property of dup-alpha7 may have important implications for the physiology and pharmacology of the alpha7 nAChR in the human brain. We used primary neocortical microglia cells isolated from the temporal cortex from neurosurgical samples to study the expression of *CHRNA7* and *CHRFAM7A*, respectively. Furthermore, we explored the effect of different alpha7 nAChR agonists on LPS-induced TNF-alpha release from these cells. While *CHRFAM7A* mRNA was expressed in mature human primary microglia cells, *CHRNA7* mRNA was almost undetectable. By contrast, human whole-cortical extracts showed higher levels of *CHRNA7* versus *CHRFAM7A* mRNA. Interestingly, the very weak partial alpha7 nAChR agonists, NS6740 and GTS-21, significantly inhibited LPS-induced TNF-alpha release from

human primary microglia cells. Effect of the same compounds, in contrast to stronger partial agonists, was observed in rat microglia cells. These data suggest that dup-alpha7 is relatively highly expressed in human microglia cells. Despite the presence of dup-alpha7 in human microglia cells, they respond significantly to partial alpha7 nAChR agonists.

Disclosures: **J.D. Mikkelsen:** A. Employment/Salary (full or part-time): Bionomics Ltd. **M.H. Sørensen:** None. **L.H. Pinborg:** None. **H.H. Hansen:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.22/G31

Topic: B.02. Ligand-Gated Ion Channels

Support: AFM grant 16026

Title: The heteromeric nicotinic receptors of the Renshaw cell are likely 3α-2β LS receptor

Authors: *B. LAMOTTE D'INCAMPS[1], D. PETERS[2], P. ASCHER[3];
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[3]Cerebral Physiol. lab., CNRS-UMR 8118, Univ. Paris Descartes, Paris, France

Abstract: At the motoneuron-Renshaw cell synapse in young mice (P5-P10) , the component of the synaptic current mediated by heteromeric nicotinic receptors (nAChRs) has a bi-exponential decay with a large fast component (time constant around 10 ms) and a small slow component (time constant around 100 ms). Because the Renshaw cell nAChRs are known to contain multiple α and β subunits, we had initially considered the possibility that the two components of the decay may involve different subunits, but found that the decay of the EPSCs remained bi-exponential in a series of knock-outs in which a given subunit had been eliminated (Lamotte d'Incamps and Ascher, 2013). We now explored the possibility that the two time constants of the decay may arise from the dual affinity of heteromeric "low sensitivity" (LS) nAChRs with a 3α-2β stoichiometry. The concentration response curve of ACh on LS nAChRs has a small foot (high affinity) and a main part (low affinity) (Harpsoe et al. 2011). Our hypothesis was that the fast decay of the EPSC may result from the fast dissociation of ACh from the low affinity sites, while the slow decay would be due to slow dissociation from the high affinity sites. This hypothesis is well supported by the effects of two compounds known to act selectively on the 3α2β stoichiometric variants of α*β* receptors, NS9283 and Zn^{++}. NS9283 has been shown to potentiate selectively LS nAChRs receptors (Timmermann et al. 2012) by slowing deactivation (Grupe et al. 2013) and transforming the concentration-response curve to ACh from a two-

component curve into a single high affinity curve (Wang et al., 2015). NS9283 (10 μM) induced a marked prolongation of the Renshaw cell EPSCs, reducing the fast component and prolonging the slow one. NS9283 also prolonged the mEPSCs. Zn^{++}, which at 100 μM has been shown to potentiate LS and inhibit HS nAChRs (Moroni et al. 2008) also prolonged the mEPSCs, as expected from the increased burst duration observed at the single channel level (Hsia et al. 2008). These results support the hypothesis that the two components of the Renshaw cell EPSC mediated by heteromeric receptors can be attributed to a single stoichiometric assembly associating 3α and 2β subunits.

Disclosures: B. Lamotte D'Incamps: None. **D. Peters:** None. **P. Ascher:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.23/G32

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant DA029127

Memory Pharmaceuticals

HiQScreen Sàrl

Title: Tropisetron enhances cognitive performance in young and aged rodents and non-human primates

Authors: *A. V. TERRY, JR[1], P. M. CALLAHAN[1], M. R. PLAGENHOEF[1], D. BERTRAND[2], S. BERTRAND[2];
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Abstract: The α7 nicotinic acetylcholine receptor (α7 nAChR) is considered a therapeutic target for neuropsychiatric disorders such as Alzheimer's disease (AD) and schizophrenia. In the present investigation we assessed the effects of the 5HT3 receptor antagonist/α7 nAChR agonist tropisetron in memory-related tasks in rats and monkeys. Administration of tropisetron (0.1-10 mg/kg, po) improved the performance of a novel object recognition (NOR) task in young Sprague-Dawley rats at a long (48 hr) delay interval and these pro-cognitive effects were blocked by the α7 nAChR antagonist methyllycaconitine (MLA). In old Fischer 344 rats, tropisetron (0.3-3 mg/kg, ip) reversed the age-related deficits of NOR performance at a shorter (5 hr) delay. In a delayed match to sample (DTMS) task, tropisetron (0.03-1 mg/kg, im) improved accuracy (% correct) at the longest (i.e., presumably the most difficult) delay intervals in both aged male and

female rhesus monkeys. In subsequent experiments, a combination of sub-effective doses of donepezil (0.1 mg/kg) and tropisetron (0.03-0.1 mg/kg) also resulted in improved DMTS accuracy at the longest delay intervals. These later experiments would appear to support the electrophysiological (priming) experiments where exposure to low concentrations of tropisetron enhanced the current evoked by acetylcholine in *Xenopus* oocytes expressing human α7 and α7β2 nAChRs (see companion poster). Collectively, these studies improve our understanding of the nAChR pharmacology of tropisetron and support the argument that the compound has the potential to improve certain domains of cognition (i.e., recognition memory, working/short memory) that are often impaired in neuropsychiatric disorders such as AD and schizophrenia.

Disclosures: A.V. Terry: None. **P.M. Callahan:** None. **M.R. Plagenhoef:** None. **D. Bertrand:** None. **S. Bertrand:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.24/G33

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant DA029127

Memory Pharmaceuticals

HiQScreen Sàrl

Title: Effects of low concentrations of tropisetron at the human α7 nAChR

Authors: *S. BERTRAND[1], P. M. CALLAHAN[2], E. NEVEU[1], A. V. TERRY, Jr[2], D. BERTRAND[1];
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Abstract: The structural similarities between the $5HT_3$ and the α7 nicotinic acetylcholine receptor (nAChR) suggest that these ligand gated ion channels share some pharmacological properties. For example, the high affinity of $5HT_3$ receptors to curare was the first demonstration of the commonality between these receptors. The observation that the $5HT_3$ receptor antagonist, tropisetron acts, albeit at high concentrations as an agonist at the α7 nAChR, provided another example of the pharmacological promiscuity between cholinergic and 5HT pathways. However, due to the difference in sensitivity these data are not sufficient to explain the pro-cognitive activity observed with low concentrations of tropisetron and its positive outcome in

schizophrenic patients.
To examine further the possible mechanisms by which low concentrations of tropisetron mediate the cognitive benefits we hypothesized that this compound might cause priming of α7 containing nAChRs. To evaluate the effects of tropisetron at α7 nAChRs we recorded the agonistic, antagonistic and priming activities of this compound at human α7 and α7β2 receptors expressed in *Xenopus* oocytes.
Data presented herein show without ambiguity that tropisetron acts as an agonist but with distinct properties at α7 and α7β2 receptors. Moreover, in priming experiments exposure to low nanomolar concentrations of this compound caused a significant enhancement of the current evoked by 40 μM acetylcholine.
Comparison of these data with results obtained from behavioral experiments (see accompanying poster) confirm that the pro-cognitive effects of tropisetron can be attributed to its activity at α7 nAChRs.

Disclosures: S. Bertrand: None. **P.M. Callahan:** None. **E. Neveu:** None. **A.V. Terry:** None. **D. Bertrand:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.25/G34

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant AA13588

NIH Grant DA030929

NIH Grant AA017072

Title: Selective activation of $(A4)_3(B2)_2$ nAChRs reduces alcohol consumption

Authors: *J. WANG[1], C. DOEBELIN[2], A. KURYATOV[3], J. LINDSTROM[3], T. M. KAMENECKA[2], P. J. KENNY[4], R. O. MESSING[1];
[1]The Univ. of Texas At Austin, Austin, TX; [2]Scripps Res. Inst., Jupiter, FL; [3]Univ. of Pennsylvania, Philadelphia, PA; [4]Icahn Sch. of Med. at Mount Sinai, New York City, NY

Abstract: Tobacco and alcohol are highly co-abused. The FDA-approved smoking cessation aid varenicline (Chantix®), a selective partial agonist of α4β2 nicotinic acetylcholine receptors (nAChRs), also reduces alcohol consumption in humans and rodents. α4β2 nAChRs can assemble in two stoichiometries: $(\alpha4)_3(\beta2)_2$ and $(\alpha4)_2(\beta2)_3$. It is unknown whether varenicline

modulates one or both stoichiometries to reduce alcohol consumption. The two stoichiometries share two high sensitivity acetylcholine (ACh) binding sites, but $(\alpha4)_3(\beta2)_2$ nAChRs have an additional low sensitivity ACh site at its unique α4/α4 subunit interface. The $(\alpha4)_3(\beta2)_2$ nAChRs have been largely overlooked because of their apparent "low" sensitivity to cholinergic agonists. In fact, low doses of ACh can activate both stoichiometries, but high doses of ACh can further activate $(\alpha4)_3(\beta2)_2$ nAChRs and evoke a 5-fold larger current. This makes $(\alpha4)_3(\beta2)_2$ nAChRs appear to be less sensitive than $(\alpha4)_2(\beta2)_3$ nAChRs after normalization to maximum evoked current. Traditional agonists and competitive antagonists all bind to the high sensitivity ACh site and cannot distinguish the two stoichiometric forms. The small molecule 3-[3-(3-pyridinyl)-1,2,4-oxadiazol-5-yl]benzonitrile (NS9283) selectively binds to the low sensitivity ACh site at the α4/α4 subunit interface and thereby enhances the sensitivity of $(\alpha4)_3(\beta2)_2$ nAChRs for ACh. However, NS9283 itself does not directly activate or inhibit activation of nAChRs. Here we used NS9283 to increase function of $(\alpha4)_3(\beta2)_2$ nAChRs and investigated their role in alcohol consumption, using C57BL/6J mice in an intermittent access 2-bottle choice procedure. We found that administration of 10 mg/kg NS9283 significantly reduced ethanol intake by 29% during the following three hours of drinking. This decrease was similar to that produced by 2 mg/kg varenicline. NS9283 potentiated activation and desensitization of $(\alpha4)_3(\beta2)_2$ nAChRs by varenicline *in vitro*. To confirm that the effect of NS9283 on ethanol consumption was through its action at $(\alpha4)_3(\beta2)_2$ nAChRs, we tested whether subthreshold doses of NS9283 and varenicline would synergize to reduce ethanol drinking. Indeed, we found that while 2.5 mg/kg NS9283 and 0.1 mg/kg varenicline had no effect, in combination at those doses, they significantly reduced ethanol consumption. These results indicate that modulating the activity of $(\alpha4)_3(\beta2)_2$ nAChRs contributes to the ability of varenciline to reduce ethanol consumption. Drugs selective for $(\alpha4)_3(\beta2)_2$ nAChRs could prove to be new therapeutics for the treatment of excessive drinking.

Disclosures: **J. Wang:** None. **C. Doebelin:** None. **A. Kuryatov:** None. **J. Lindstrom:** None. **T.M. Kamenecka:** None. **P.J. Kenny:** None. **R.O. Messing:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.26/G35

Topic: B.02. Ligand-Gated Ion Channels

Support: the Danish Council for Independent Research (DFF-4183-00246)

Danish Strategic Research Council (COGNITO)

the Lundbeck Foundation

Title: Perinatal nicotine exposure transiently increases NACHO protein levels in the rat frontal cortex

Authors: *M. S. THOMSEN[1], F. WICHERN[1], M. C. GONDRÉ-LEWIS[2], J. D. MIKKELSEN[3], H. B. HANSEN[3];
[1]Univ. of Copenhagen, Kobenhavn O, Denmark; [2]Dept. of Anat., Howard Univ. Col. of Med., Washington D.C., WA; [3]Neurobio. Res. Unit, Univ. Hosp. Copenhagen, Rigshospitalet, Copenhagen, Denmark

Abstract: Maternal smoking during and after gestation can have detrimental effects on the neuronal development of the offspring. The underlying molecular mechanisms involved in this effect are not completely understood, but it is believed that the major pharmacologically active chemical found in tobacco smoke, namely nicotine, is responsible via activation of the nicotinic acetylcholine receptors (nAChRs) in the developing brain.
NACHO (from the *TMEM35* gene) protein was recently shown to bind and influence the functionality of the α7 (alpha7, alpha 7) and α4β2 nicotinic acetylcholine receptors (nAChRs, Gu et al., Neuron, 2016, PMID: 26875622). We hypothesized that expression of the NACHO protein underlie the nAChR upregulation after perinatal nicotine exposure.
Nicotine (4mg/kg/day) was delivered through osmotic minipumps to SPRD rats from gestational day 7 until postnatal day (PND) 21, when the offspring was weaned. The male offspring were sacrificed at PND 7, 21, or 60 and the frontal cortex was subjected to western blotting. We found that nicotine significantly increased NACHO protein levels at PND7 and PND21 (2-fold increase, $P<0.05$ at both time points), but not at PND60. By contrast, adult exposure to nicotine (0.4 mg/kg, s.c., twice per day for 7 days), had no effects on NACHO protein levels in the frontal cortex. This indicates that exposure to nicotine during early development transiently increases NACHO levels in the brain. This increase in NACHO may explain nicotine-induced upregulation of nAChRs during development.
In summary, our results suggest that NACHO is sensitive to nicotine during early development, but that the effect is transient, and in adulthood NACHO levels are no longer affected by nAChR agonists. This suggests that NACHO protein is not involved in agonist-induced nAChR upregulation in adulthood.

Disclosures: **M.S. Thomsen:** None. **F. Wichern:** None. **M.C. Gondré-Lewis:** None. **J.D. Mikkelsen:** A. Employment/Salary (full or part-time): Employed at Bionomics Ltd. **H.B. Hansen:** None.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.27/G36

Topic: B.02. Ligand-Gated Ion Channels

Support: Innovation Fund Denmark (Cognito)

Title: The alpha7 nicotinic acetylcholine receptor regulator chaperone (NACHO) is expressed in high levels in the hippocampus and prefrontal cortex of the rat

Authors: *H. B. HANSEN[1], F. WICHERN[2], M. S. THOMSEN[2], J. D. MIKKELSEN[1,3]; [1]Neurobio. Res. Unit, Copenhagen Univ. Hosp., Copenhagen, Denmark; [2]Dept. of Drug Design and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark; [3]Bionomics Ltd., Thebarton, South Australia, Australia

Abstract: The alpha7 nicotinic acetylcholine receptor (alpha7 nAChR) is one of the most abundant nAChR subtypes in the CNS and considered a potential drug target for alleviating cognitive deficits. The nicotinic acetylcholine receptor regulator chaperone (NACHO) has recently been reported to interact with the alpha7 nAChR subunit, resulting in markedly enhanced cell surface expression of mature alpha7 nAChRs. It is important to reveal the regional distribution of NACHO in the brain, because the presence of NACHO in the cell is essential for functionality of the alpha7 nAChR. In this study, we used immunohistochemistry to map the distribution of NACHO immunoreactivity in the rat brain. While NACHO immunopositive cells were observed predominantly in the cell soma, fiber structures were also labelled. NACHO immunoreactive neurons were found in high numbers in the hippocampus. The prefrontal cortex was particularly labelled, as compared to other cortical regions. Intense labelling was also observed in the ventromedial and arcuate nuclei of the hypothalamus, as well as in the lateral habenula. Moderate immunostaining was localized to the amygdala, lateral septum, bed nucleus of the stria terminalis and thalamus. The substantia nigra pars compacta showed low expression of NACHO. In conclusion, NACHO distribution corresponds to a major extent, but not fully, to brain regions with high alpha7 nAChR binding levels in the rat. NACHO immunoreactivity was also represented in brain regions with high alpha4beta2 nAChR expression. This suggests that NACHO plays an important role in regulating both alpha7 and alpha4beta2 nAChR activity.

Disclosures: H.B. Hansen: None. **F. Wichern:** None. **M.S. Thomsen:** None. **J.D. Mikkelsen:** A. Employment/Salary (full or part-time): Bionomics, Thebarton, South Australia, Australia.

Poster

500. Nicotinic Acetylcholine Receptors in the Brain: Physiology and Function

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 500.28/G37

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Funding from SRF for 2013-2016 (KK & TF)

Grant-in Aid for Scientific Research (15K07969) from the Ministry of Education, Science, Sports and Culture (C) of Japan (KK, TF and MM)

Title: Role for α7 nicotinic acetylcholine receptors in naïve T cell differentiation into regulatory T cell

Authors: *K. KAWASHIMA[1], M. MASHIMO[2], T. FUJII[2], Y. MORIWAKI[3], H. MISAWA[4], S. ONO[5];
[1]Kitasato Univ. Sch. of Pharm., Tokyo, Japan; [2]Pharmacol., Doshisha Col. of Libeeral Arts, Fac. of Pharmaceut. Sci., Kyoto, Japan; [3]Pharmacol., Keio University, Fac. of Pharm., Tokyo, Japan; [4]Pharmacol., Keio Univ. Sch. of Pharm., Tokyo, Japan; [5]Immunol., Osaka Ohtani Univ. Sch. of Pharm., Osaka, Japan

Abstract: We have shown that $CD4^+$ T cells have the ability to synthesize acetylcholine (ACh) by choline acetyltransferase (ChAT), and that immunological activation of T cells via the T-cell receptor (TCR) stimulation increases ChAT mRNA expression and the ACh content. Furthermore, immune cells including T and B cells, macrophages and dendritic cells express various subtypes of both muscarinic and nicotinic ACh receptors (mAChRs and nAChRs, respectively). These findings suggest that ACh synthesized and released from $CD4^+$ T cells plays a role in the regulation of immune responses acting in autocrine and/or paracrine fashion via mAChRs and nAChRs on various immune cells. Among nAChR subtypes, α7 nAChRs down-regulate the synthesis and release of TNF-α in macrophages activated with LPS. Moreover, we showed that production of antigen-specific IgG_1 antibody and the pro-inflammatory cytokines, such as TNF-α, IFN-γ and IL-6, is elevated in α7 nAChR-deficient (α7-KO) compared with the wild-type C57BL/6J (WT) mice, suggesting a regulatory role for α7 nAChR in immune responses. In the present study, we investigated the role for α7 nAChRs in the differentiation of FoxP3-expressing regulatory T cells (Tregs) using α7-KO and WT mice. The spleen cells were stimulated with anti-TCR and anti-CD28 mAbs in the presence or absence of GTS-21 (3-30 μM), a partial α7 nAChR agonist, for 5 days in 24-well plate. At the end of the culture, we analyzed Tregs induction by the expression of CD4, CD25 and Foxp3 using flow cytometry. GTS-21 at 30 μM up-regulated significantly the generation of Tregs in WT spleen cells, while GTS-21 did not change the induction of Tregs in α7-KO spleen cells. The results show that GTS-21 up-regulates Tregs differentiation via the action on α7 nAChRs, and they support the notion that ACh released

from activated T cells acts on α7 nAChRs in naïve T cells and promotes Tregs induction leading to suppression of immune responses. Collectively, these findings suggest that a failure of Treg induction in α7-KO mice is responsible for elevated production of antigen-specific IgG_1 antibody, at least in part.

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Poster

501. Sodium Channels

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 501.01/G38

Topic: B.04. Ion Channels

Support: NSFC31271153

Title: Dynamic regulation of astrocyte cholesterol level and its impact on transmembrane ion gradient and glutamate transport

Authors: ***Z. YE**[1,2], Y. ZHOU[2], B. YE[2], T.-T. SHI[2], L. WANG[2], B. R. RANSOM[1];
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Abstract: Oxidative stress has been reported to reduce glutamate transport, with suggested mechanisms ranging from lipid peroxidation to modification of transporter properties. However, little attention has been paid to the potential impact of oxidative stress on transmembrane ion gradients, particularly the Na^+ gradient, the chief driving force for Na^+-dependent glutamate transporters. We now report that oxidative stress caused deregulation of intracellular [Na^+] ($[Na^+]_i$) leading to reduced astrocyte glutamate transport, specifically reduced uptake. we also found significant reduction of cholesterol levels in astrocytes. Using cultured mouse astrocytes, oxidative stress was achieved by application of hydrogen peroxide (20 to100 uM). Meanwhile, application of the cholesterol binding compound MβCD also led to dose- and time-dependent increases in astrocyte $[Na^+]_i$, reversible upon removal of MβCD and inhibited by application of water soluble cholesterol. Accompanying the restoration of $[Na^+]_i$ in astrocytes after MβCD removal, cholesterol level also recovered even in the absence of precursors for cholesterol synthesis, indicating abundant intracellular storage of the precursors in astrocytes and their ability to be rapidly utilized. In summary, oxidative stress and MβCD both reduced cholesterol levels, presumably via different mechanisms. These mechanisms could operate in parallel to increase astrocyte $[Na^+]_i$, inhibiting glutamate uptake and increasing glutamate release from

astrocytes. Our results indicate that astrocytes dynamically regulate their cholesterol content and that this regulation can affect one of their major functions, the uptake of the neurotransmitter glutamate.

Disclosures: **Z. Ye:** None. **Y. Zhou:** None. **B. Ye:** None. **T. Shi:** None. **L. Wang:** None. **B.R. Ransom:** None.

Poster

501. Sodium Channels

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 501.02/G39

Topic: B.04. Ion Channels

Support: Rehabilitation Research Service and Medical Research Service, Department of Veterans Affairs

Title: Ankyrin G is an anchor for myosins and voltage-gated sodium channels in the nervous system

Authors: ***B. DASH**, C. HAN, F. DIB-HAJJ, P. SHAH, S. G. WAXMAN, S. D. DIB-HAJJ; Neurol., Yale Univ. Dept. of Neurol., West Haven, CT

Abstract: Ankyrin G (AnkG) serves as an adaptor protein that links membrane proteins to the underlying cytoskeleton in neurons. Functionally it is comprised of a membrane binding domain (MBD), a spectrin binding domain and a C-terminal regulatory domain that also contains a death domain. Various proteins including integral membrane proteins (such as voltage-gated sodium channels, sodium-calcium exchangers, sodium-potassium ATPase; etc.), signaling molecules and cytoskeletal components interact with each of these domains. This enables AnkG assemble the axon initial segment (AIS); and organize and stabilize protein networks. In this work, using immunochemical methods, we show for the first time that Class II, V and VI myosins interact with AnkG. Specifically, all the three isoforms of class II non-muscle myosin [i.e., non-muscle myosin IIA, IIB and IIC, respectively know as myosin heavy chain 9 (myh9), myosin heavy chain 10 (myh10) and myosin heavy chain 14 (myh14)], myosin Va (MyoVa) and myosin VI (MyoVI) interact with both the 270- and 190-KDa isoforms of AnkG. Preliminary results indicate that the MBD of AnkG is involved in its interaction with myosins. We hypothesize that this novel interaction of AnkG and myosins is important for formation of the AIS; and trafficking and targeting of voltage-gated sodium channels to functionally distinct axonal and somatodendritic compartments.

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Poster

501. Sodium Channels

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Program#/Poster#: 501.03/G40

Topic: B.04. Ion Channels

Support: NSF OCE1040597

NSF OCE1459235

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Ida Russell Cades Foundation

Title: Multiplicity of sodium channel genes in crustaceans

Authors: *D. K. HARTLINE[1], P. H. LENZ[2], V. RONCALLI[3];
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Abstract: Multiplicity of function at the transcriptional level within a gene family is commonly achieved through gene duplication followed by divergent adaptive evolution or by alternative splicing within a single gene. While vertebrates achieve extensive diversity of their voltage-gated sodium channels (Na_Vs) though gene duplication, most invertebrates, and particularly insects, obtain diversity through alternative splicing within a single gene designated "Na_V1." Surprisingly, several Na_V1s have been reported recently in a planktonic crustacean, the calanoid copepod *Calanus finmarchicus* (designated CalfiNa_V1.1, 1.2 and 1.3). Using a canonical *Drosophila melanogaster* sequence as query (DmNa_V1), we utilized the availability of an increasing number of transcriptomic databases to search for multiplicities of Na_V1 genes in other crustaceans. Databases from the Malacostraca, Cladocera and Maxillopoda consistently yielded Na_V1 sequences with good homology to DmNa_V1, including multiple putative splice variants in several cases, similar to the pattern present in insects. In addition to an insect-like Na_V1 with multiple splice variants ($Na_V1.1$), we found evidence in several other copepods of additional Na_V1 genes, with patterns of substitutions and insertions/deletions not easily explained by a splice-variant hypothesis. Using Calfi $Na_V1.2$ or 1.3 as query sequences we have failed so far to find close homologs of these genes, or indeed of comparable multiplicities, in available databases from arthropods and invertebrates outside of the Copepoda. We suggest that

exceptional selective pressures on copepods have resulted in the evolution of a greater diversity of Na_V1 genes than has been found among the rest of the Arthropoda, suggesting unexpected roles for this channel group in the taxon. Support: NSF OCE1040597 (UH), OCE1459235 (UH), ABI1062432 (Indiana U) and the Ida Russell Cades Foundation (Honolulu).

Disclosures: **D.K. Hartline:** None. **P.H. Lenz:** None. **V. Roncalli:** None.

Poster

501. Sodium Channels

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Topic: B.04. Ion Channels

Support: AHA Grant #13SDG16990083

Title: Estimating ion channel models with prior knowledge

Authors: *M. A. NAVARRO, A. SALARI, L. MILESCU;
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Abstract: Ion channel gating mechanisms can be complex and difficult to extract from experimental data. A solution is to apply parameter constraints, which reflect prior knowledge or tested hypotheses and reduce model complexity and speed up computation. Soft constraints balance the existing knowledge with the new experimental data and limit the parameter search engine to a smaller space of more acceptable values. In contrast, hard constraints enforce a mathematical relationship involving one or more parameters of the model. These constraints can be formulated as an invertible transformation between a set of model parameters and a set of "free" parameters. Each constraint reduces the number of free parameters by one. Linear constraints, such as microscopic reversibility or scaling between sequential transitions, can be conveniently obtained with the singular value decomposition. Here, we show how this method can be generalized to implement arbitrary linear constraints. We also show how to make these constraints depend on arbitrary model parameters. This can be applied, for example, to enforce allosteric constraints where the allosteric factor itself is a free parameter. Furthermore, we demonstrate how slack variables can be used to implement inequality constraints.

Disclosures: **M.A. Navarro:** None. **A. Salari:** None. **L. Milescu:** None.

Poster

501. Sodium Channels

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Topic: B.04. Ion Channels

Support: 15ZR1424300

2014CB910302

2014CB910301

Title: Upregulation of voltage-gated sodium channels by Fyn kinase

Authors: Y. LI[1], T. ZHU[1], H. YANG[1], T. XU[1], Y. YU[2], S. D. DIB-HAJJ[3,4], S. G. WAXMAN[3,4], *X. CHENG[1];
[1]Discipline of Neurosci. and Dept. of Anatomy, Histology and Embryology, [2]Inst. of Med. Sci. and Dept. of Pharmacol. and Chem. Biol., Shanghai Jiao Tong Univ. Sch. of Med., Shanghai, China; [3]Dept. of Neurol. and Ctr. for Neurosci. and Regeneration Res., Yale Univ. Sch. of Med., New Haven, CT; [4]Dept. of Neurol. and Rehabil. Res. Ctr., Veterans Affairs Connecticut Healthcare Syst., West Haven, CT

Abstract: Voltage-gated sodium channels (VGSCs) play essential roles in initiation and propagation of action potentials in excitable cells, and are subjects to many protein modifications such as phosphorylation, ubiquitylation, and proteolysis. Nine genes have been identified to encode VGSC α subunits, generating Nav1.1 to Nav1.9 channels. Previously it has been reported that Fyn kinase, a member of Src tyrosine kinase family, interacted with Nav1.2 and Nav1.5, and differentially altered channel properties. Here, using constitutively-active form (Fyn^{CA}) or dominant-negative form (Fyn^{DN}) of Fyn kinase, we investigated the effects of Fyn kinase on the expression and gating properties of Nav1.2, Nav1.6, Nav1.7, and Nav1.8 channels. Fyn^{CA} significantly elevated the protein levels of all four VGSC subtypes expressed in both HEK 293 and ND7/23 cells, when compared with cells transfected with Fyn^{DN}. Voltage-gated sodium currents were recorded from HEK 293 or ND7/23 cells transiently transfected with sodium channels plus Fyn^{CA} or Fyn^{DN} using whole-cell recordings. In HEK 293 cells, Fyn^{CA} significantly increased Nav1.2 and Nav1.7 current densities, when compared with cells transfected with Fyn^{DN}. In contrast, Fyn^{CA} had no effect on current densities of Nav1.6 and Nav1.8 channels recorded from transfected ND7/23 cells, despite it increased the total protein levels of Nav1.6 and Nav1.8. Small yet significant shifts in channel gating induced by Fyn^{CA} were observed, with no consistent changes among the four sodium channel subtypes. Our results demonstrate that Fyn kinase could regulate the expression of VGSCs, which might enable it to participate in the physiological and pathological functions of VGSCs.

Disclosures: **Y. Li:** None. **T. Zhu:** None. **H. Yang:** None. **T. Xu:** None. **Y. Yu:** None. **S.D. Dib-Hajj:** None. **S.G. Waxman:** None. **X. Cheng:** None.

Poster

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Topic: B.04. Ion Channels

Support: ICM P09-015-F (BNI)

ICM P10-001-F (CENEM)

CONICYT Master Scholarship (2013-22131877)

Title: Functional inactivation of adult rat hippocampus through neosaxitoxin intrahippocampal injection

Authors: ***J. GALINDO**, N. LAGOS W, J. VALDES;
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Abstract: The Neosaxitoxin (NeoSTX) is a marine toxin which blocks the voltage-gated sodium channels, producing an arrest of action potential propagation. The effects of NeoSTX are well characterized in the peripheral nervous system. However, there is no information about its effects in the central nervous system (CNS).
This research gives the first approach to elucidate the effects of NeoSTX over the CNS. With the purpose of evaluating this; adult rats were injected with NeoSTX at different doses (2.5 ng y 5.1 ng) in the CA3 region of the hippocampus and then the animals were tested for performance in a hippocampal-dependent spatial memory task. Also, we evaluated the effect of the toxin on the neuronal activity through immunohistochemical analysis by using the marker of neuronal activity c-Fos in the affected zone. Finally, we evaluated the presence of neuronal damage in the toxin injection zone, using histological analysis.
The results showed that NeoSTX can inactivate the hippocampus trough the blocking of voltage-gated sodium channels. The inactivation of the hippocampus cause a disruption in the spatial memory capacity and a decrease in the hippocampal neuronal activity, essayed by c-Fos immunoreactivity, which lasts up to 48 hours, after the toxin injection. The functionality of the hippocampus and its neuronal activity was fully recovered after 72 hours of treatment, without damage to the nervous tissue. These results demonstrated that the intrahippocampal

administration of NeoSTX generates a long lasting and reversible inactivation of this structure without neuronal damage.

Disclosures: **J. Galindo:** None. **N. Lagos W:** None. **J. Valdes:** None.

Poster

501. Sodium Channels

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Topic: B.04. Ion Channels

Support: Brain Research Foundation Grant Number BRFSG-2015-05

Graduate Assistance of Areas in National Need (GAANN) Grant Number P200A150059

Title: The role of cellular adhesion molecules on voltage-gated sodium channel distribution and action potential generation at the axon initial segment

Authors: *S. ALPIZAR[1], M. HOPPA[2];
[2]Biol., [1]Dartmouth Col., Hanover, NH

Abstract: Voltage gated sodium channel (VGSC) types 1.2 and 1.6 are recruited to the axon initial segment (AIS) following the enrichment of the scaffolding protein AnkyrinG during development. Previous studies have proposed that an ankyrinG-binding motif within an intracellular loop of the VGSC is sufficient for targeting VGSCs to the AIS. However, despite the shared homology of an intracellular loop known to bind AnkyrinG, the two VGSC types segregate at the AIS and exhibit differing distributions. Thus, the mechanisms enabling preferential targeting of VGSCs within the AIS remain unknown. Also uniquely localized at the AIS are cellular adhesion molecules (CAMs) such as Neuronal Cellular Adhesion Molecule, Neurofascin-186, and the sodium channel beta subunits. While these molecules are known for their functions in neurite extension, axon formation, and neuron-glial interactions, the effect that they may have on VGSC trafficking at the AIS in unknown. Our aim is to identify a potential role for CAMs to segregate VGSCs within the AIS and elucidate the functional consequence of their loss on action potential (AP) initiation.
Using primary rat hippocampal cultures, we combined localization measurements of fluorescently tagged VGSCs with genetic ablation of select CAMs using shRNA. These findings were combined with AP measurements that allow for firing and waveform analysis using synaptophysin- GCaMP, a genetically encoded Ca^{2+} indicator localized to synaptic vesicles that

provides accurate detection of single action potentials. Titrating the current until a lack of calcium influx (firing) is observed allowed us to measure the firing threshold of neurons with ablated CAMs. The loss of select CAMs altered the localization of VGSCs, but did not change trafficking of AnkyrinG. Additionally, not all changes in VGSC distribution correlated with AP initiation properties. Therefore, proper localization of VGSCs and AP initiation are coordinated in part by CAMs.

Disclosures: S. Alpizar: None. **M. Hoppa:** None.

Poster

501. Sodium Channels

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Program#/Poster#: 501.08/G45

Topic: B.04. Ion Channels

Support: Rehabilitation Research Service and Medical Research Service, Department of Veterans Affairs

Connecticut Stem Cell Research Grants Program

Title: Engineering iPSCs from inherited erythromelalgia patients with Nav1.7 mutations to study the firing properties and drug responsiveness of human sensory neurons

Authors: *Y. YANG, S. D. DIB-HAJJ, M. ESTACION, J. HUANG, S. G. WAXMAN; Neurol., VA CT Healthcare Syst., West Haven, CT

Abstract: Inherited erythromelalgia (IEM), a severe pain syndrome characterized by episodes of intense burning pain, is caused by mutations in sodium channel Nav1.7 which is preferentially expressed in sensory and sympathetic neurons. IEM provides a genetic model of chronic pain. Most IEM patients do not respond to pharmacotherapy, and it is critically important to understand the molecular mechanisms behind the pattern of pharmacoresponsiveness of IEM patients, which may facilitate the development of personalized treatment for chronic pain based on genetic information. Indeed, using a precision medicine approach, we matched a medication (carbamazepine) to two IEM patients with the Nav1.7-S241T mutation based on our genomic analysis and functional profiling. In vitro models might also contribute to the precision medicine approach to chronic pain. Induced pluripotent stem cells (iPSCs) are promising tools to model human neurological diseases, and with CRISPR/Cas9 genome-editing technology, accurate modification of specific genes within iPSCs is possible. To develop a “chronic pain in a dish” disease model, we have obtained blood samples from IEM patients with Nav1.7-S241T mutation

and generated iPSCs. We have differentiated these iPSCs into sensory neurons for functional and pharmacotherapeutic studies. As Nav1.8 is a primary marker for sensory neurons, we have also engineered a Venus fluorescent tag fused with Nav1.8 gene in the genome of H9 embryonic stem cells and iPSCs. Isogenic controls are being produced by correcting the S241T mutation in the iPSCs. These engineered iPSCs will serve as valuable tools to study the firing properties and drug responsiveness of human sensory neurons with disease-causing mutations.

Disclosures: Y. Yang: None. **S.D. Dib-Hajj:** None. **M. Estacion:** None. **J. Huang:** None. **S.G. Waxman:** None.

Poster

501. Sodium Channels

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Support: ERC Advanced Grant 268548

Austrian Science Fund P24909-B24

Title: Energy-efficient action potential signaling in inhibitory interneuron axons

Authors: H. HU[1], *P. JONAS[2];
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Abstract: Fast-spiking, parvalbumin-expressing GABAergic interneurons (PV+ interneurons) play a key role in several microcircuit functions, such as feedforward and feedback inhibition, high-frequency network oscillations, and temporal encoding of information in the brain (Hu et al., 2014, Science 345:1255263). For all of these functions, the fast initiation, propagation, and termination of axonal action potentials (APs) is critically important. However, previous studies suggested that short APs are metabolically highly expensive (Carter and Bean, 2009, Neuron 64: 898-909). Together with the AP high frequency in PV+ interneurons in vivo, this suggests that a substantial part of the total energy budget of the brain may be used by processes associated with APs of PV+ interneurons. However, previous conclusions regarding AP energy efficiency in PV+ interneurons were made based on analysis of Na^+ channels in interneuron somata. Whether these conclusions hold for PV+ interneuron axons, where the majority of voltage-gated Na^+ channels is located, remains an important, but entirely open question. To estimate the energy efficiency of APs in interneuron axons, we performed confocally targeted subcellular patch-

clamp recordings in interneuron axons. Na^+ currents were recorded in outside-out patches using APs as voltage-clamp commands at near-physiological temperature (34-37^{o}C). Our results indicated a Na^+ inflow of 1.2 ± 0.5 pmole cm^{-2} per AP in the interneuron axon (n=11). This value is comparable to those of other cell types, including axons of glutamatergic principal neurons (1.76 pmole cm^{-2}, Alle et al., 2009, Science 325:1405-1408). Thus, in terms of total Na^+ inflow, PV+ interneurons appeared to be as energy efficient as other types of neurons. We next compared the total Na^+ entry during the AP with the theoretical minimum (Na^+ entry ratio) in the PV+ interneuron axons. Although the entry ratio in the interneuron axon (2.6 ± 0.3, n=21) appeared to be higher than in pyramidal neurons, it was substantially lower than the value of 4 previously obtained in the squid giant axon. Computer modeling of AP initiation and propagation in PV+ interneurons indicated an inflow of 581 × 10^8 Na^+ ions during a single AP. With the stoichiometry of the Na^+-K^+-ATPase, this would correspond to 194 × 10^8 ATP molecules per AP. Thus, the energy requirement per AP is significantly smaller in PV+ interneurons than in layer 5 neocortical pyramidal neurons (800 × 10^8 ATP molecules per AP, Hallermann et al., 2012, Nat Neurosci 15:1007-1014). In conclusion, AP signaling in GABAergic interneuron axons is more energy-efficient than previously thought.

Disclosures: H. Hu: None. **P. Jonas:** None.

Poster

501. Sodium Channels

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Topic: B.04. Ion Channels

Support: BBSRC and Pfizer CASE PhD studentship BB/J500872/1 (completed)

Title: Temperature dependence of neurophysiological properties of IB4-positive and -negative mouse DRG neurons

Authors: *M. A. MIS[1], S. DIB-HAJJ[1], E. B. STEVENS[2], A. D. RANDALL[3], S. G. WAXMAN[1];
[1]Neurol., Yale Univ., West Haven, CT; [2]Pain and Neurosci. Res. Unit, Pfizer, Cambridge, United Kingdom; [3]Med. Sch., Univ. of Exeter, Exeter, United Kingdom

Abstract: Temperature can exert a variety of effects on voltage-gated ion channels, thus affecting cellular excitability. In pain-related electrophysiology, a sum of currents differently affected by temperature may result in dissimilar nociceptor properties at room and near-physiological temperatures. However, temperature effects on voltage-gated Na^+ channels and on

the output of DRG neuron firing are not well understood. The current project is focused on studying the effect of temperature on the neurophysiological properties of IB4-positive and -negative mouse dorsal root ganglia (DRG) neurons and the TTX-sensitive and -resistant Na^+ current expressed in these neurons. DRG neurons from 4-week-old male C57Bl/6 mice were cultured for up to 48 hours. Voltage- and current-clamp recordings were performed on IB4-positive and -negative small-diameter neurons at room temperature (RT, ~22°C) and 35°C. Preliminary IB4-positive data revealed resting membrane potential (RMP) hyperpolarization, a significant drop in input resistance and a decrease in firing frequency at 35°C. Interestingly, the decrease in firing frequency was only apparent at potentials close to the RMP. For cells that were held at more hyperpolarized pre-stimulus potentials, firing frequency increased at 35°C, but decreased at RT. These alterations could be partly driven by differential temperature-dependent changes in biophysical properties of the TTX-sensitive and TTX-resistant Na^+ current expressed in these cells. Specifically, while the peak TTX-sensitive Na^+ current increased significantly at 35°C, there was no change in the peak TTX-resistant Na^+ current. Consequently, the ratio of TTX-sensitive to TTX-resistant Na^+ current was found to be different at RT and 35°C. We also observed hyperpolarized shifts of the activation and inactivation curves for both types of current. IB4-positive and -negative DRG neurons were shown to be neurobiochemically distinct and have been suggested to convey information about distinct noxious stimuli and maintain different types of pain. Since the expression pattern of voltage-gated ion channels, including Na^+ channels, varies between IB4-positive and -negative mouse DRG neurons, it is expected that the two groups of cells will react to an increase in temperature in disparate ways. This question is currently being addressed by ongoing detailed characterization of the temperature dependence of IB4-negative mouse DRG neurons.

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Poster

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Support: Medical Research Service, Dept of Veterans Affairs

Rehabilitation Research Service, Dept of Veterans Affairs

Yale University from Convergence Pharmaceuticals

Title: A gain-of-function mutation in $Na_v1.6$ in a case of trigeminal neuralgia

Authors: ***B. S. TANAKA**[1,2,3], P. ZHAO[1,2,3], F. B. DIB-HAJJ[1,2,3], V. MORISSET[4], S. TATE[4], S. G. WAXMAN[1,2,3], S. D. DIB-HAJJ[1,2,3];
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Abstract: Idiopathic trigeminal neuralgia (TN) is a debilitating pain disorder characterized by episodic unilateral facial pain along the territory of branches of the trigeminal nerve. Human painful disorders other than TN have been linked to gain-of-function mutations in voltage-gated sodium channels ($Na_V1.7$, $Na_V1.8$ and $Na_V1.9$) which are expressed in the peripheral nervous system (PNS). Gain-of-function mutations in $Na_V1.6$, which is expressed in myelinated and unmyelinated CNS and PNS neurons and supports neuronal high-frequency repetitive firing, have been linked to epilepsy but not to human pain disorders. Here, we describe a 64-year-old female who presented with evoked and spontaneous paroxysmal unilateral facial pain, and carried a diagnosis of TN. Magnetic resonance imaging showed unilateral neurovascular compression, consistent with classical TN and pain in areas innervated by the second branch of the trigeminal nerve. Genetic analysis as part of a phase 2 clinical study in patients with trigeminal neuralgia conducted by Convergence Pharmaceuticals Ltd (NCT01540630) revealed a previously undescribed de novo missense mutation in $Na_V1.6$ (c.A406G; p.Met136Val). Whole-cell voltage-clamp recordings show that the Met136Val mutation significantly increases peak current density (1.5-fold) and resurgent current (1.6-fold) without detectable effects on gating properties. Current-clamp studies in trigeminal ganglion (TRG) neurons showed that Met136Val increased the fraction of high-firing neurons, lowered the current threshold and increased the frequency of evoked action potentials in response to graded stimuli. Our results demonstrate a novel $Na_V1.6$ mutation in TN, and show that this naturally-occurring mutation potentiates transient and resurgent sodium currents and leads to increased excitability in TRG neurons. We suggest that this gain-of-function mutation of $Na_V1.6$ may exacerbate the pathophysiology of vascular compression and contribute to TN.

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Poster

501. Sodium Channels

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Topic: B.04. Ion Channels

Support: ANR-2011-BSV4-001-1

Title: Anchoring of ankyrin G to its membrane partners drives the establishment and maintenance of the axon initial segment

Authors: *C. LETERRIER, N. CLERC, F. RUEDA-BORONI, A. MONTERSINO, B. DARGENT, F. CASTETS;
CRN2M, Marseille Cedex 15, France

Abstract: In neurons, the axon initial segment (AIS) concentrates multiple types of ion channels (sodium, potassium, calcium) and cell adhesion molecules (CAM). These membrane proteins are associated with scaffolding proteins and cytoskeletal components at the AIS. Many studies have established the crucial role of the scaffolding protein ankyrin G (ankG) in the recruitment of membrane components at the AIS. However, the possible reciprocal role of these membrane components in ankG targeting and stabilization has been largely ignored so far. In cultured hippocampal neurons and cortical organotypic slices, we found that shRNA-mediated knockdown of voltage-gated sodium channels VGSCs led to an impaired formation and maintenance of the AIS, independently of their ion channel activity. A similar decrease in ankG concentration was found after knockdown of the AIS CAM neurofascin-186. In both cases, this decrease could be rescued by the expression of a recombinant VGSC, but also by a minimal construct containing the VGSC Nav1.2 ankyrin binding domain and a membrane anchor (mABD). These results show that anchoring of ankG to its membrane partners, in particular VGSCs, is a key step for the establishment and stabilization of the AIS assembly. Moreover, overexpressing mABD in mature neurons led to ankG delocalization, suggesting that ankG association to VGSCs may occur before its insertion at the AIS.

Disclosures: **C. Leterrier:** None. **N. Clerc:** None. **F. Rueda-Boroni:** None. **A. Montersino:** None. **B. Dargent:** None. **F. Castets:** None.

Poster

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Topic: B.04. Ion Channels

Support: BRFSG-2015-05

Title: Sodium channel beta2 subunits control action potential propagation fidelity

Authors: *I. CHO, M. HOPPA;
Dartmouth Col., Hanover, NH

Abstract: The arrival of an action potential at a presynaptic terminal dictates both calcium channel opening and the driving force for calcium entry, which are powerful levers in sculpting synaptic efficacy. Excitatory hippocampal neurons contain highly ramified axons (~200 branch points) that form a large number of en passant boutons (~8,000). When an axon dilates or branches this will cause an increased electrical load due to impedance mismatches that can alter the biophysical fidelity of the action potential (AP), or in extreme cases stop transmission. This impedance mismatch can be addressed at the subcellular level by endowing larger diameter axonal structures with a higher density of ion channels to compensate for the increased electrical load. At present, we have little idea about how cells regulate the density and subcellular distribution of ion channels to ensure AP delivery and signal fidelity. This question has been particularly difficult to study in hippocampal neurons due to the fine structures of distal axonal branches that preclude detailed electrophysiological analysis. Here we have used a combination of optogenetic indicators to make subcellular measurements of both membrane potential and calcium influx at nerve terminals to study sodium channel distribution across the axon of cultured hippocampal neurons. We demonstrate that very low (~6 nM) application of tetrodotoxin (TTX) revealed selective branch point failures suggesting a heterogeneous distribution of sodium channels. To determine a molecular mechanism, we ablated all four sodium channel beta subunits ($Na_v\beta$s) to investigate a potential role for these subunits for functionally enrichment voltage-gated sodium channels at branch points and synaptic boutons. Ablation of $Na_v\beta2$ uniquely caused high (~50%) branch point failure rates in AP propagation as well as a loss of signal fidelity with large variability observed in synaptic AP amplitudes across the axonal arborization. Finally, we show that geometrical dilations, especially synaptic bouton densities, alter the biophysical fidelity of propagating action potentials. Our results suggest that $Na_v\beta2$ regulates the function of Na^+ channels at branch points to compensate for the morphological properties of axons, ensuring signal transmission fidelity. Given the fact that synaptic transmission is highly sensitive to changes Ca^{2+} influx controlled by the biophysical

properties of the AP, this finding reveals a new important role for $Na_v\beta$ subunits to control the spread of excitability in the nervous system.

Disclosures: I. Cho: None. **M. Hoppa:** None.

Poster

501. Sodium Channels

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Topic: B.04. Ion Channels

Title: *In vitro* biophysical and pharmacological characterization of four human Nav1.7 mutations associated with pain phenotypes

Authors: *D. LIU[1], N. RAMPAL[1], B. GRUBINSKA[2], P. ROSE[3], J. GINGRAS[2], B. MOYER[1]; [1]Neurosci., AMGEN, Inc., Thousand Oaks, CA; [2]Neurosci., [3]Biologics, AMGEN, Inc., Cambridge, MA

Abstract: Genetic and functional studies have demonstrated that $Na_v1.7$ plays a critical role in human pain signaling: gain-of-function mutations underlie pain syndromes such as inherited erythromelalgia (IEM), paroxysmal extreme pain disorder (PEPD), and small fiber neuropathy (SFN), and loss-of-function mutations underlie channelopathy-associated congenital insensitivity to pain (CIP). $Na_v1.7$ is considered an attractive target for the development of next-generation analgesics to reduce pain. In this study, we characterized the biophysical properties of four human $Na_v1.7$ sodium channel mutations associated with disease (L858H-IEM, M1627K-PEPD, I720K-SFN and R896Q-CIP) in parallel with wild-type (WT) human $Na_v1.7$ expressed in U2OS cells using the BacMam expression system and evaluated by manual patch clamp electrophysiology. Compared to WT $Na_v1.7$, the L858H mutant left-shifted the $V_{1/2}$s of activation and slow-inactivation (30 second pre-pulse duration) curves by 17 mV and 15 mV respectively, indicating increased activation and slow inactivation; the M1627K mutant right-shifted the $V_{1/2}$ of fast-inactivation (1 second pre-pulse duration) by 18 mV, indicating reduced fast inactivation; the I720K mutant right-shifted the $V_{1/2}$s of activation and slow-inactivation by 10 mV and 9 mV respectively, indicating reduced activation and slow inactivation. The R896Q mutant did not produce robust macroscopic currents under control conditions. However, when co-incubated with 500 uM mexiletine for 48 hours, sizable sodium currents >800 pA were recorded from cells expressing R896Q mutant channels after mexiletine washout, suggesting that mexiletine may function as a pharmacogical chaperone for this non-stop codon-based CIP mutation. Sodium currents recorded from WT and the four $Na_v1.7$ mutations were completely blocked by a $Na_v1.7$-selective small molecule inhibitor. qRT-PCR and Western blot analyses

revealed that mRNA and full-length protein were present in U2OS cells transduced with BacMam viruses harboring either WT or the individual $Na_v1.7$ mutations. $Na_v1.7$ inhibitors may reduce pain not only in individuals expressing WT $Na_v1.7$, but also in individuals harboring gain-of-function $Na_v1.7$ channelopathy mutations.

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Poster

501. Sodium Channels

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Topic: B.04. Ion Channels

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Canadian Foundation for Innovation

Title: Temperature dependent changes in neuronal dynamics in a novel SCN1A mutation

Authors: *C. H. PETERS[1], R. E. ROSCH[2], E. HUGHES[3], P. C. RUBEN[1];
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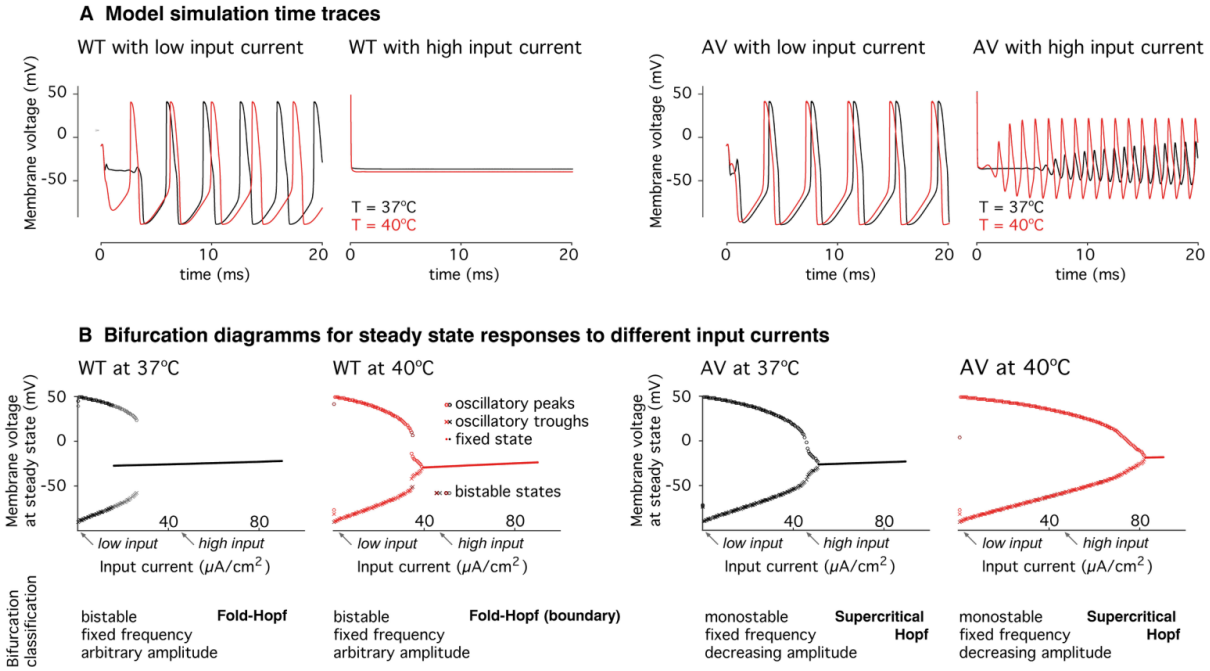
Abstract: Dravet syndrome is a severe SCN1A-mutation associated epilepsy. It is characterised by prolonged seizures, typically provoked by fever. We describe the evaluation of an SCN1A mutation in a child with early-onset temperature-sensitive seizures.The patient carries a heterozygous missense variant (c3818C>T; pAla1273Val) in SCN1A which encodes the $Na_V1.1$ brain sodium channel. We compared the functional effects of the variant vs. wild type $Na_V1.1$ using patch clamp recordings from channels expressed in Chinese Hamster Ovary Cells at different temperatures (32, 37, and 40°C). The variant channels produced a temperature-dependent destabilization of activation and fast inactivation. Implementing these empirical abnormalities in a computational model produces a higher threshold for depolarization block in the variant, particularly at 40°C, suggesting a failure to autoregulate at high-input states. These results reveal direct effects of abnormalities in $Na_V1.1$ biophysical properties on neuronal

dynamics. They illustrate the value of combining cellular measurements with computational models to integrate different observational scales (gene/channel to patient).



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Poster

501. Sodium Channels

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UK Medical Research Council MR/M006948/1

Title: Gating pore currents in novel S4 arginine mutant $Na_V1.4$ channels: myotonia and block by Heriaeus melloteei spider toxin

Authors: ***M. THOR**[1], D. KUZMIN[1], S. DURRAN[1], E. MATTHEWS[1], R. SUD[1], A. A. BERKUT[2], S. SCHORGE[1], M. G. HANNA[1], D. M. KULLMANN[1], A. A. VASSILEVSKI[2], R. MÄNNIKKÖ[1];

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Abstract: Gain of function mutations in the skeletal muscle sodium channel $Na_V1.4$ (*SCN4A*) cause myotonia and hyperkalemic periodic paralysis (hyperPP). In contrast, hypokalemic periodic paralysis (hypoPP) is caused by mutations that introduce gating pore currents through the voltage sensing domain by neutralizing arginine residues in the fourth transmembrane segment, S4. We identified the novel *SCN4A* mutation R222Q located in S4 of domain I in a patient with myotonia. The patient also carries a mutation in the chloride channel ClC-1, associated with recessive myotonia congenita. A different mutation in the same *SCN4A* residue, R222W, was found in a patient with hypoPP. Mutant sodium channels were expressed in HEK293 cells and *Xenopus* oocytes, and studied by whole-cell patch clamp and two-electrode voltage clamp, respectively. The ability of a gating modifier toxin Hm-3 to block gating pore currents was also studied using molecular dynamics and electrophysiological recordings. Both mutant channels produced gating pore currents. The R222Q mutation also caused a hyperpolarizing shift in the voltage dependence of activation. Gating pore currents from both R222 mutant channels were blocked by Hm-3 ($IC_{50} \approx 3$ μM). Hm-3 failed to block gating pore currents caused by homologous mutations in domain II and III, or by mutagenesis of R219 in domain I. We have performed molecular dynamics simulations to look into the potential molecular basis of these differences. Gating pore currents of R222W channels are consistent with hypoPP, while the enhanced activation of R222Q mutant channel is consistent with myotonia. Gating modifier toxins may be useful as gating pore blockers in hypoPP therapy, especially due to their putative domain- and isoform-specific mechanism of action.

Disclosures: M. Thor: None. **D. Kuzmin:** None. **S. Durran:** None. **E. Matthews:** None. **R. Sud:** None. **A.A. Berkut:** None. **S. Schorge:** None. **M.G. Hanna:** None. **D.M. Kullmann:** None. **A.A. Vassilevski:** None. **R. Männikkö:** None.

Poster

501. Sodium Channels

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 501.17/H3

Topic: B.04. Ion Channels

Title: Investigation of background Na^+ channels important for pacemaking in the nigral dopamine neurons

Authors: *S. HAHN, M. PARK;
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Abstract: Dopamine neurons in the midbrain are slow pacemakers. Without any afferent inputs they produce regular APs. However, it is still not known what ion channels are responsible for pacemaking in dopamine neurons. Among many candidates, unidentified Na^+-permeable leak channels appear to be one of major channels for driving pacemaker activity. Since resting membrane potentials of midbrain dopamine neurons are maintained between -55-45 mV far from the equilibrium potential of K^+(E_k), there could be a conductance responsible for persistent depolarization of membrane potential. Therefore, we have investigated background Na^+-permeable ion channels responsible for maintaining membrane potential depolarized in nigral dopamine neurons, using Ca^{2+} measurement and patch-clamp techniques. Since extracellular Ca^{2+} and Na^+ can play a key role in determining resting leak conductances, we examined whether extracellular Ca^{2+} and Na^+ influence membrane potentials, firing rates, and inward currents in dopamine neurons. Lowering $[Ca^{2+}]_e$ from 2.0 to 0.5 mM increased the Na^+ leak inward current and heavily affected spontaneous firing rates. A nonselective cation channel blocker for TRPC channels, SKF-96365, did not completely block background Na^+ conductances. Despite usage of TTX and Cs^+ which block Na_v and K_v channels, the component of Na^+ leak conductances survived. RT-PCR showed the presence of mRNA for NALCN, a background Na^{2+} leak channel, in dopamine neurons. These results suggest that there could be a TTX- and Cs^+-resistant Na^+ leak channel controlled by $[Ca^{2+}]_e$ and that NALCN could play an important role in the pacemaking of the midbrain dopamine neurons.

Disclosures: **S. Hahn:** None. **M. Park:** None.

Poster

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Support: NIH/NIMH Grant R01 MH095995-A1

NSF Grant DMS 1320910

Title: Kernel-based classification of a GSK3-centric protein network at the axonal initial segment

Authors: ***W.-C. HSU**[1], P. NEGI[3], S. PRASAD[4], D. LABATE[3], F. LAEZZA[2];
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Abstract: The axonal initial segment (AIS) in neurons integrates electrical inputs from neurites and converts it into an electrical output through the action of voltage-gated sodium (Nav) channels, providing a mechanism to regulate excitability and plasticity. Nav channels are just one of the many elements in the Nav macromolecular complex, which contains proteins with important regulatory and structural roles such as ankyrin-G, neurofascin, spectrin, and intracellular fibroblast growth factors (iFGFs, FGF11-14). Dysfunction in any of these components has been linked to serious psychiatric and neurological disorders in both humans and in animal models. By applying a nonlinear kernel-based Support Vector Machine (SVM) classifier on a large data set of high-resolution confocal images, we discovered several novel phenotypes in downstream AIS complex proteins caused by perturbation of a GSK3-centric kinase network, including alterations in spectrin/Nav channels as well as neurofascin/spectrin expression and co-localization upon treatment with Wee1 and GSK3 inhibitors. This methodology is a novel, powerful approach towards interrogation of large imaging data sets to better understand the phenotypic significance of intracellular signaling pathways controlling the molecular determinants of neuronal excitability in the brain.

Disclosures: **W. Hsu:** None. **P. Negi:** None. **S. Prasad:** None. **D. Labate:** None. **F. Laezza:** None.

Poster

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Topic: B.04. Ion Channels

Support: NIH R01MH095995 (FL)

King Saud University, Saudi Arabia, Ph.D. scholarship (MA)

King Saud University, Saudi Arabia, Ph.D. scholarship (TA)

Title: FGF14 is a master organizer of the beta-IV spectrin complex at the axonal initial segment and the paranodal junction in cortical regions

Authors: *M. A. ALSHAMMARI[1], T. K. ALSHAMMARI[1], M. N. NENOV,[2], F. SCALA[2], F. LAEZZA[2];
[1]Pharmacol. and Toxicology, Col. of Pharmacy, King Saud Univ., Riyadh, Saudi Arabia;
[2]pharmacology and toxicology, Univ. of Texas Med. Br., Galveston, TX

Abstract: The macromolecular complex of proteins at the axonal initial segment (AIS) and at paranodal junctions is a critical determinant of neuronal excitability. By providing the scaffolding matrix for anchoring the voltage-gated Na+ channel (Nav) complex and its accessory proteins, these two subcellular compartments are subject to a tight control in neurons. Loss of such homeostatic regulation leads to neurodegeneration and cell death, common across many brain disorders. The intracellular fibroblast growth factor 14 (FGF14) is a functionally relevant component of the AIS that through binding to the intracellular C-tail of the Nav channel controls the channel trafficking and function via direct modulation and through kinase activity. In translational studies, mutations and/or changes in FGF14 gene expression level have been identified in ataxias and recently in schizophrenia raising interest in the mechanism of action of this molecule in animal models. Here, we show that genetic deletion of *Fgf14* leads to a selective loss and disruption of the beta- IV spectrin and of Caspr/neurexin IV complex in hippocampus and in myelinated cortical fibers. Correlative changes are found in Nav1.6 immunoreactivity and in functional properties of neurons. With patch-clamp electrophysiology in acute brain slices, we show that CA1 pyramidal neurons of *Fgf14*$^{-/-}$ mice exhibit increased action potential threshold and lower firing frequency. These results provide new insights into the biology of FGF14 further supporting its pivotal role as a structure-function organizer of the macromolecular complex of Nav channels and as a brain disease-associated risk factor

Disclosures: M.A. Alshammari: None. **T.K. Alshammari:** None. **M.N. Nenov,:** None. **F. Scala:** None. **F. Laezza:** None.

Poster

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Title: Altered $Na_v1.2$ inactivation kinetics in a case of brain malformation, refractory seizures, and death

Authors: N. ELIA[1], S. DHILLON[1], E. DELA CRUZ[1], A. MONTALVO[1], X. XIONG[1], P. CHEN[1], S. HIROSE[2], T. L. KLASSEN[3], *D. H. FELDMAN[1], C. J. SAUNDERS[4], C. LOSSIN[1]; [1]Neurol., UC Davis, Sch. of Med., Sacramento, CA; [2]Pediatrics, Fukuoka Univ., Fukuoka, Japan; [3]Univ. of British Columbia, Vancouver, BC, Canada; [4]Children's Mercy Hosp., Kansas City, KS

Abstract: Voltage-gated sodium channels (Na_v channels) initiate action potentials in excitable tissues, which makes them strategic participants in excitation disorders of skeletal muscle, heart, and brain. For many of these conditions, the basic Na_v channel dysfunction has been established at the molecular level. Much less is known about how Na_v channels contribute to development, in particular that of the central nervous system. While it is recognized that neural activity shapes synaptic interactions and the development of patterns of connectivity, the details are poorly understood. We here report on the functional abnormalities of one particular Na_v channel mutant that was found in an infant with brain anomalies, epilepsy, and death at age 2 months. The Na_v channel in question, $Na_v1.2$, has established ties to epilepsy. Its connection to brain development, however, is less clear. Whole-genome sequencing of patient DNA had revealed no alterations of significance, with the exception of an *SCN2A* variant (p.Arg1626Gln or R1626Q) that predicted neutralization of a positively charged arginine in the voltage sensor of the fourth homologous domain (D4/S4). To characterize the functional impact of this exchange, we stably transfected HEK cells with either a wild-type or an R1626Q-mutant $Na_v1.2$ plasmid construct and conducted whole-cell voltage clamping. Several channel parameters were relatively unaffected, including the current-voltage relationship and the voltage dependence of activation, as well as the voltage dependence of both, fast and slow inactivation. Mutant channels, however, tended to require longer hyperpolarization to recover from fast inactivation, and, more clearly, R1626Q channels displayed markedly slowing of the fast inactivation kinetics. These biophysical changes might lead to an overall gain-of-function and augmented excitability, which could, at least partly, underlie the seizures observed in the patient. Rodent modeling will help determine how alterations of ion channel kinetics can affect complex developmental processes to produce a severe, and ultimately, life-incompatible phenotype.

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Poster

501. Sodium Channels

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Topic: B.04. Ion Channels

Support: NIH NS053422

Title: Palmitoylation modifies biophysical properties of voltage-gated sodium channel 1.6.

Authors: *Y. PAN[1,2], Y. XIAO[2], T. R. CUMMINS[2];
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Abstract: Palmitoylation has been identified as a reversible post-translational lipid modification that dynamically regulates protein trafficking and membrane association. It was previously shown that brain voltage-gated sodium (Nav) channels are subjected to palmitoylation and Nav1.2 channels exhibit different functional properties in different palmitoylation states. We investigated how palmitoylation modulates the biophysical properties of Nav1.6, one of the most abundant Nav in the central nervous system. Nav1.6 play a major role in neuronal excitability, underlying the initiation and propagation of action potentials. Our aim was to identify palmitoylation sites that potentially can be pharmacologically modified in this channel. We transiently expressed the mouse isoform of Nav1.6 in ND7/23 cell line and performed whole-cell patch clamp. We discovered that interrupting palmitate incorporation with 2-Br-palmitate, a palmitate analog, results in a hyperpolarizing shift of the voltage-dependence of inactivation and slower kinetics of recovery from inactivation in the channel. To identify the palmitoylation sites responsible for these functional alternations, we substituted two cysteines (C1169, C1170), predicted to be palmitoylated in Nav1.6, with alanines and found that the double cysteine mutant exaggerated the hyperpolarizing shift we observed with 2-Br-palmitate treatment, but did not display slower recovery from inactivation. We concluded that 1) the functional properties of Nav1.6 can be regulated by its palmitoylation states; 2) C1169, C1170 in Nav1.6 are potential palmitoylation sites that could be modulated to change channel gating properties and potentially to alter neuronal excitability; 3) palmitoylation at different cysteine residues may exert distinct regulatory effects in channel biosynthesis, membrane association, gating and pharmacological properties.

Disclosures: Y. Pan: None. **Y. Xiao:** None. **T.R. Cummins:** None.

Poster

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Gulf Coast Consortia NIGMS Grant T32 GM089657 (SRA)

PhRMA Foundation (FL)

Title: Modulation of the FGF14:Nav1.6 channel interaction through short peptide-based probes

Authors: *S. R. ALI, Z. LIU, M. NENOV, F. SCALA, T. JAMES, A. SINGH, H. CHEN, J. ZHOU, F. LAEZZA;
Dept. of Pharmacol. & Toxicology, Univ. of Texas Med. Br. at Galveston, Galveston, TX

Abstract: Voltage-gated sodium (Nav) channels are responsible for initiation and propagation of action potentials in neurons. Dysregulation of specific Nav channel isoforms is found across a wide range of brain disorders associated with motor and cognitive disabilities. Unfortunately, currently available probes and drugs targeting Nav channels are directed against highly conserved domains of the channels and as such lack specificity. The macromolecular complex of Nav channels is a source of less conserved protein-protein interaction (PPI) interfaces that represent a unique opportunity for the design of potentially novel chemical probes to interrogate the brain circuitry and future more targeted leads to treat Nav channelopathies. The intracellular fibroblast growth factor 14, FGF14, is a biologically relevant component of the neuronal Nav channel complex controlling gating, stability and trafficking of native Nav channels. Through a monomeric interaction with the intracellular C-terminal tail of Nav channel α subunits, FGF14 binds and modulates the activity of Nav channels in an isoform-specific manner. Using molecular modeling and *in silico* docking as guidance, we designed a peptidomimetic fragment based on the FGF14:Nav1.6 interface and validated its activity using a combination on *in vitro* and *ex vivo* assays including the split-luciferase assays surface plasmon resonance and whole-cell patch clamp electrophysiology in heterologous systems and in nucleus accumbens (NAc) acute brain slices. Overall, our data indicate that the newly synthesized peptidomimetic fragment may compete with FGF14 for binding to Nav1.6 channels suppressing neuronal firing in medium

spiny neurons in the NAc. This study will lay ground work for the development of a new class of PPI-based allosteric modulators of Nav channels with potential therapeutic values for a variety of brain disorders.

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Poster

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Topic: B.04. Ion Channels

Support: Grants from the Rehabilitation Research Service and Medical Research Service, Department of Veterans Affairs

Title: A gain-of-function Nav1.9 mutation causes early-onset familial episodic pain

Authors: ***C. HAN**[1,2], Y. YANG[1,2], R. H. TE MORSCHE[3], J. P. H. DRENTH[3], J. M. POLITEI[4], S. G. WAXMAN[1,2], S. D. DIB-HAJJ[1,2];
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Abstract: Sodium channel Nav1.9 is preferentially expressed in peripheral nociceptive somatosensory neurons and visceral afferents, and myenteric neurons. Gain-of-function mutations of Nav1.9 have been linked to human painful disorders, including painful peripheral neuropathy and familial episodic pain and in a case of painless channelopathy. Here we report a novel Nav1.9 mutation, an arginine 222 substitution by histidine (R222H) in the domain I S4 segment, identified in a family with episodic pain. The affected family members experienced pain mainly in distal extremities including joints and gastrointestinal disturbances with onset in early childhood, with pain severity diminishing with age (less after 16-18 years). Multistate modeling study suggested that mutation from arginine to histidine disrupts the interactions between this residue and negatively charged residues at physiological pH, which would destabilize the channel's resting and early activation states and favor channel opening. Voltage-clamp analysis demonstrated that the mutation hyperpolarizes channel activation by 6.4 mV compared with wild-type Nav1.9 channels. Current-clamp analysis showed that the R222H mutant channels render dorsal root ganglion neurons hyperexcitable, including depolarized

resting potential, reduced current threshold, increased evoked firing and increased spontaneous firing. Taken together, our observations show that the new gain-of-function mutation in Nav1.9 which can confer hyperexcitability on peripheral sensory neurons underlies episodic pain. This data expands the spectrum of inherited pain disorders related to mutations of sodium channel Nav1.9.

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Poster

501. Sodium Channels

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Topic: B.04. Ion Channels

Title: Distinct pre- and postsynaptic localizations of Na_v1 subtypes in rat hippocampus areas CA1 and dentate gyrus.

Authors: *J. PLATHOLI[1], K. JOHNSON[1], K. HEROLD[1], T. MILNER[2], H. C. HEMMINGS, Jr.[1];
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Abstract: Voltage-gated Na^+ channels (Na_v) initiate and propagate action potentials crucial for neuronal excitability and signal transduction. Plasticity of synapses can be regulated by forward and back-propagating action potentials leading to long-term potentiation (LTP) and long-term depression (LTD) depending on frequency and strength of stimulation. Abnormal Na_v activity is also central to the pathophysiology of epileptic seizures. How Na_v channels mediate excitability for these dual synaptic functions is unclear; cellular and regional differences in Na_v subtype expression and localization could underlie both roles. Axonal and somatodendritic Na^+ currents vary in their gating properties suggesting different subtypes are preferentially localized with distinct physiological functions. Three major subtypes (Na_v 1.1, 1.2, 1.6) are highly expressed in adult mammalian brain, but their neuroanatomical organizations in hippocampal subregions with distinct with layer-specific differences in excitatory and inhibitory inputs are unknown. To delineate differences in Na_v channel expression relevant to their diverse neurophysiological functions, roles in disease and pharmacological effects, we utilized light and electron microscopy to determine the spatial distribution of Na_v 1.1, Na_v 1.2, and Na_v 1.6 in hippocampal subfields of area CA1 and dentate gyrus (DG) in rat. All three subtypes were localized postsynaptically in area CA1 and were associated with asymmetric or excitatory synapses. Interestingly, in the DG

Na_v 1.1 was localized presynaptically, while Na_v 1.2 and Na_v 1.6 showed no preference but all subtypes were also associated with asymmetric synapses.

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Poster

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Support: NIH R01 MH095995 (FL)

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Gulf Coast Consortia NIGMS T32 GM089657 (SRA)

Title: Structure-function determinants of the FGF14:Nav1.6 channel complex

Authors: ***A. K. SINGH**, S. ALI, F. LAEZZA;
Pharmacol. & Toxicology (MRB Build.), Univ. of Texas Med. Br. (UTMB), Galveston, TX

Abstract: The voltage-gated Na^+ (Nav) channel complex provides the basis for electrical excitability in the brain and has been linked to the etiology of brain-related channelopathies. In native conditions, the Nav channel is regulated by a number of accessory proteins including fibroblast growth factor 14 (FGF14), a member of the intracellular FGFs family. Through protein:protein interactions (PPI) FGF14 controls biophysical properties of Nav1.6-encoded currents such as peak transient current, voltage-dependence of activation, steady-state inactivation and fast inactivation. In previous studies, we used a combination of molecular modeling, split-luciferase assay, and patch-clamp electrophysiology to map and validate critical hot-spots at the FGF14:Nav1.6 channel complex interface. Our results indicate that either the $FGF14^{V160A}$ or the $FGF14^{K74A/I76A}$ mutations are sufficient to abolish FGF14-dependent regulation of peak transient Na^+ currents and voltage-dependence of activation and steady-state inactivation of Nav1.6, but that only V160A with a concomitant alanine mutation at Y158 can impede FGF14-dependent modulation of the channel fast inactivation. Here, we apply biophysical measurements to parse out the structural role of these critical amino acid residues in regulating binding to the Nav1.6 C-tail. Intrinsic fluorescence spectroscopy (IFS), Surface Plasmon Resonance and circular dichroism were used to characterize the binding properties of FGF14 wild type and mutants to the Nav1.6 C-terminal tail. IFS confirmed significant binding

reduction of $FGF14^{V160A}$ to the Nav1.6 C-tail indicating a key role of this residue in mediating PPI. Altogether these studies identify critical structure-function determinants of the macromolecular complex of Nav1.6 channels that might provide guidance for therapeutic development against brain disorders.
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Disclosures: **A.K. Singh:** A. Employment/Salary (full or part-time): Full, University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555. Other; Research Scientist II. **S. Ali:** None. **F. Laezza:** None.

Poster

501. Sodium Channels

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Topic: B.04. Ion Channels

Support: CNPq

Funcap

Title: Experimental diabetes mellitus preferentially alters the myelinated fibers of the vagus nerve and increases the TTX-S sodium currents of nodose ganglia

Authors: *K. S. SILVA-ALVES[1], F. W. FERREIRA-DA-SILVA[2], T. A. ALVES-FERNANDES[3], A. N. COELHO-DE-SOUZA[3], J. H. LEAL-CARDOSO[3];
[2]Superior Inst. of Biomed. Sci., [1]State Univ. of Ceara, Fortaleza, Brazil; [3]Superior Inst. of Biomed. Sci., State Univ. of Ceará, Fortaleza, Brazil

Abstract: Diabetic neuropathy is the most common diabetes mellitus (DM) complication and can affect many nerves of the organism. The vagus nerve (VN) is the longest cranial nerves and innervates several visceras of the body. Since DM is already known to affects firstly long nerves, this study aimed to investigate the alterations produced by DM on VN and nodose ganglion (GN) neurons. Wistar rats were randomly divided into control (CONT) and diabetic (DB) groups. The DB group was induced by administration of streptozotocin (65 mg/kg, via i.p.) at 8^{th} week of life, while CONT group received solely sodium citrate buffer (vehicle). Rats were sacrificed at the end of 12^{th} week and VN and NG were dissected. The fuction of myelinated and unmyelinated VN axons was evaluated through the extracellular recording of compound action potential (CAP). The patch clamp technique was used to record Na^+ currents from dissociated neurons of

NG. In 12th week, the glycemic level of CONT and DB groups were 112.8 ± 4.0 and 456.9 ± 15.9 mg/dL (n = 22). The CAP of VN myelinated fibers presented 3 distinct waves and the conduction velocities of each in CONT were 32.4 ± 3.6, 13.9 ± 1,4 e 5.4 ± 0.5 m/s and the duration of the 1st and 2nd waves were 0.4 ± 0.1 e 0.5 ± 0.1 ms, respectively. For DB group, there was a reduction of about 50% of conduction velocity (17.6 ± 1.3 and 7.6 ± 0.1 m/s) and an increase of 150% in the duration (1.1 ± 0.3 and 1.4 ± 0.3 ms) of the 1st and 2nd CAP components compared to the CONT group. For VN unmyelinated fibers in DB group there were no alterations in the conductibility and excitability parameters but when challenged with tetrodotoxin (TTX), VN unmyelinated fibers become more sensitive. The IC_{50} for 2nd and 3rd waves in CONT were 979.5 ± 30.6 and 879.2 ± 20.2 nM and for DB group were 662.8 ± 104.3 e 718.2 ± 179.8 nM. The patch clamp recording in dissociated neurons of NG showed an increase in normalized amplitude of total Na^+ current (INa^+) from 71.0 ± 8.3 in CONT to 113.4 ± 16.7 pA/pF (n = 11) in DB group. There was a shift of ~ 10 mV to hyperpolarized potentials in Na^+ current activation and steady state inactivation curves. Voltage separation of total INa^+ in their sensitive (TTX-S) and resistant (TTX-R) components, showed that only TTX-S INa^+ conductance was increased in DB group neurons from 39.0 ± 3.9 pA/pF in CONT to 115.7 ± 20.9 pA/pF (n = 8) in DB group. In conclusion, streptozotocin-induced DM preferentially alters myelinated fibers of VN, increases the amplitude and conductance of total INa^+ and INa^+ TTX-S of NG. Also, there was a shift in voltage-dependence of activation and inactivation curves and these changes in Na+ current in NG partially explain the alteration seen in VN fibers.

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Poster

501. Sodium Channels

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Topic: B.04. Ion Channels

Support: FAPESP 2012-09426-1

CNPQ 470745-2012-6

Title: The persistent sodium current increases near-threshold input resistance and membrane time constant via a negative slope conductance

Authors: *C. C. CEBALLOS[1], A. C. ROQUE[1], R. M. LEAO[2];
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Abstract: A change of the input resistance (R_{in}) of the neuron involves a change in the membrane conductances by opening and closing of ion channels. In passive membranes, i.e., membranes with only linear leak conductances, the increase or decrease of these conductances leads to a decrease or increase of the R_{in} and the membrane time constant (τ_m). However, the presence of subthreshold voltage dependent currents can produce non-linear effects generating deviations from this relationship, especially the contradictory effect of negative conductances, as produced by the sodium-persistent current (I_{NaP}), on the R_{in}. In this work we aimed to analyze experimentally and theoretically the impact of the negative conductance produced by I_{NaP} on R_{in}. Experiments of whole-cell patch-clamp conducted in CA1 hippocampus pyramidal cells from brain slices showed a paradoxical voltage-dependent decrease of the R_{in} and the τ_m in subthreshold membrane potentials close to the firing threshold after the perfusion with TTX, which inhibits I_{NaP}. This effect is postulated to be a result of the negative slope conductance in the subthreshold region produced by this conductance. The analysis of the experimental data, together with simulations found that the slope conductance of I_{NaP} is negative for subthreshold membrane potentials and its magnitude is voltage dependent in the same range observed for the voltage-dependence of R_{in} and τ_m. The injection of an artificial I_{NaP} using dynamic-clamp in the presence of TTX restored the R_{in} and τ_m to its original values. Additionally the injection of an artificial leak current with a negative conductance in the presence of TTX restored the R_{in} and τ_m as the artificial I_{nap} did. On the other hand, the injection of an artificial leak current with a positive conductance in the presence of TTX had no effect on the R_{in} and τ_m. We conclude that I_{NaP} increases the R_{in} and τ_m by the negative slope conductance observed in its non-monotonic I-V relationship. These results demonstrate that the effect of I_{nap} on R_{in} and τ_m is stronger in potentials near the firing threshold, which could potentiate the temporal summation of the EPSPs increasing their temporal integration and facilitating action potential firing. Because of its negative slope conductance, I_{NaP} is more effective in increasing excitability near threshold than a depolarizing leak current.

Disclosures: C.C. Ceballos: None. **A.C. Roque:** None. **R.M. Leao:** None.

Poster

501. Sodium Channels

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 501.28/H14

Topic: B.04. Ion Channels

Title: Pyrethroid insecticide effects on spontaneous electrical activity in neural networks are consistent with effects on voltage gated sodium channels (VGSCs) and dependent on time, concentration, and structure

Authors: J. D. STRICKLAND[1,2], C. GRANT[4], J. ROSS[1], *W. D. ATCHISON[3], T. J. SHAFER[4];
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Abstract: Pyrethroid (PYR) insecticides are used in a wide range of commercial products to control insect pests. They induce insecticidal and toxicological effects by disrupting VGSC kinetics thus altering neural excitability. Effects on VGSC function are related to the presence or absence of a cyano group on the PYR. Previous studies examined effects of 2 PYRs, deltamethrin and permethrin, on neural network function. However, characterization of effects on network function by other PYRs is lacking, and examination of changes across time and concentration are limited. Here, changes in spontaneous neural network function following exposure to 13 PYRs and 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p,p'-DDT) were monitored over 10 min intervals for 40 min using multi-well microelectrode array (mwMEA) plates. Baseline activity (40 min) of mixed cultures (neurons and glia) from rat cortex on mwMEAs was recorded prior to exposure to non-cytotoxic concentrations (0.03-40 μM) of PYRs. After 40 min, all compounds except allethrin decreased neural network mean firing rates (MFR) at 40 μM. However, effects on MFR were time- and concentration-dependent, and generally aligned with chemical structure. Cyano-containing (deltamethrin, β-cyfluthrin, fenpropathrin, esfenvalerate, cypermethrin) PYRs generally decreased MFR in a time-dependent manner, completely inhibiting MFR at 40 μM. Resmethrin, permethrin, allethrin, bifenthrin, tetramethrin, prallethrin, and p,p'-DDT, which lack a cyano group, generally increased MFR in a time- and concentration-dependent manner to 2-3 fold over control values at 3-10 μM. Increases in MFR were either present at 0-10 min (bifenthrin, permethrin, prallethrin, and p'p'-DDT) or at 20-30 min (tetramethrin, allethrin, S-bioallethrin, and resmethrin). Tefluthrin, which lacks a cyano group, caused a response similar to cyano-containing compounds. Further, although they decreased MFR at higher concentrations, esfenvalerate (0.1-0.3 μM) and cypermethrin (0.3-1.0 μM) also increased MFR 2-3 fold. The results demonstrate that PYR effects on neural networks are both time- and concentration-dependent, and differ based on the presence of a cyano group, consistent with their effects on VGSCs (This abstract does not reflect EPA policy.)

Disclosures: J.D. Strickland: A. Employment/Salary (full or part-time): Axion Biosystems. **C. Grant:** None. **J. Ross:** A. Employment/Salary (full or part-time): Axion Biosystems. **W.D. Atchison:** A. Employment/Salary (full or part-time): Axion Biosystems. **T.J. Shafer:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Axion Biosystems.

Poster

501. Sodium Channels

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 501.29/H15

Topic: F.09. Thirst and Water Balance

Support: NIH NHLBI RO1 HL115208 (Teruyama)

Title: Effect of dietary salt intake on ENaC in vasopressin synthesizing neurons in the rat supraoptic nucleus

Authors: *K. SHARMA, M. HAQUE, R. GUIDRY, R. TERUYAMA;
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Abstract: An accumulating body of evidence suggests that epithelial Na^+ channels (ENaCs) in the brain play a significant role in the regulation of blood pressure. All three ENaC subunits (α, β, and γ) were demonstrated in vasopressin (VP) synthesizing magnocellular neurons in the hypothalamic supraoptic (SON) and paraventricular nuclei, indicating ENaC affects blood pressure by modulating VP release. Our previous study showed that ENaC mediates a Na^+ leak current that affects the steady state membrane potential of VP neurons. The present study was conducted to assess the effect of dietary NaCl intake on ENaC regulation and activity in VP neurons. Male rats were fed either a high NaCl, a NaCl deficient diet, or a control diet for 7-10 days, and the relative difference in ENaC subunit mRNAs in the SON was assessed by real-time RT-PCR. A high NaCl diet caused a significant increase in β- and γENaC mRNAs in the SON, while expression of αENaC did not change. A change in the subcellular distribution of αENaC immunoreactivety in magnocellular neurons in response to dietary NaCl intake was also observed using confocal microscopy. The labeling in the magnocellular neurons from the high NaCl diet group was distinctively concentrated towards the plasma membrane in all rats tested, while those from the NaCl deficient and control groups were mainly dispersed in the cytoplasm with no distinct labeling at the plasma membrane. These findings imply that high dietary NaCl intake induces not only gene expression of ENaC subunits, but also translocation of ENaC to the plasma membrane. Electrophysiological experiments were performed to assess the effect of dietary salt intake on ENaC activity in VP neurons. The ENaC current was obtained from the difference in the steady state currents before and after the application of the ENaC blocker, benzamil. The mean amplitude of whole-cell ENaC currents and the current density were significantly larger in VP neurons from the high NaCl diet group than those from the control diet group. Collectively, these findings suggest that the regulation and activity of ENaC in VP neurons are modulated by dietary salt intake. Moreover, the modulation of ENaC in VP neurons in response to dietary salt intake is likely to have a significant contribution to the regulation of blood pressure.

Disclosures: **K. Sharma:** None. **M. Haque:** None. **R. Guidry:** None. **R. Teruyama:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 502.01/H16

Topic: B.05. Transporters

Support: NSFC31271153

Title: system xc (xct) glutamate transport is dynamically regulated in glioma cells

Authors: ***L. WANG**[1], Y. ZHOU[1], N. JIA[1], Q.-F. LU[1], T.-T. SHI[1], Z.-Q. WANG[1], B. R. RANSOM[2], Z. YE[2];
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Abstract: Glioma cell lines may exhibit significant differences in glutamate transport and metabolism. For example, some glioma cells can transition between net glutamate release and net glutamate uptake. Net glutamate transport is determined by the balance of glutamate release and glutamate uptake, and the two main systems for these opposed functions are EAAT1-mediated glutamate uptake and system Xc (xCT)-mediated glutamate release. System Xc exchanges cystine and glutamate, and normally exports glutamate. In contrast, EAAT1 is a Na^+-dependent glutamate transporter and normally imports glutamate. The variability seen in glutamate management in glioma cells related to changes in both xCT and EAAT1. We found that xCT expression was highly variable among glioma cell lines. Expression and function further varied due to other factors including cell density (cell-cell contact),and culture medium pH. Intracellular redox status and PKC activity levels played key roles in regulating expression of both EAAT1 and xCT. While PKC activation (by PMA) inhibited EAAT1 expression, it dramatically increased xCT mRNA transcription and increased the expression of xCT and related proteins. Exposure to N-acetylcysteine (NAC) increased EAAT1 expression while it simultaneously decreased xCT expression, thus facilitating the conversion from net glioma cell glutamate release to net glutamate uptake. Redox status and glutamate handling status also significantly affected glutathione (GSH) levels and GSH synthetic enzymes. Our results indicated complex and bi-directional regulation of xCT and EAAT1 expression and function in glioma cells. These factors may prove important in refining clinical management of gliomas.

Disclosures: **L. Wang:** None. **Y. Zhou:** None. **N. Jia:** None. **Q. Lu:** None. **T. Shi:** None. **Z. Wang:** None. **B.R. Ransom:** None. **Z. Ye:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 502.02/H17

Topic: B.05. Transporters

Title: Neuroprotective properties of novel positive allosteric modulators of EAAT2

Authors: R. FALCUCCI, O. MEUCCI, J. M. SALVINO, *A. C. FONTANA;
Pharmacol. and Physiol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Excitatory amino acid transporters (EAATs) play a crucial role in the removal of synaptic glutamate to maintain extracellular concentrations below excitotoxic levels. Glutamate-mediated excitotoxicity has been associated with a number of diseases/conditions, including traumatic brain injury (TBI), stroke, Amyotrophic Lateral Sclerosis, HIV-associated neurocognitive disorders (HAND) among others. Compounds that increase the activity of EAAT2, the major glutamate transporter in the brain, could have therapeutic potential for neuroprotection. Previous work has identified molecular determinants implicated in EAAT2 transport stimulation. Additionally, a hybrid structure based approach has identified selected hit structures that interact with this region. Some of these novel compounds were shown to be selective positive allosteric modulators (PAMs) of the function of EAAT2, in glutamate transport assays in transfected COS-7 cells and in glial cultures. In this work, we have characterized the effects of novel PAMs of EAAT2 in a bilaminar culture model, in which rat cortical neurons are cultured in the presence of a glial feeder layer. To examine potential neuroprotective effects of these PAMs, cultures were subjected to excitotoxic and oxidative stress insults. Specifically, excitotoxic insults were performed with the application of 1, 10, 50 or 100μM L-glutamate, and oxidative stress with the application of 10, 50 or 100μM H_2O_2. Prolonged insults were carried out for 24 h, while acute insults were carried on for 20 min, in the presence or absence of a glial layer. Compounds were applied after the insults and neuronal survival was assessed by MAP-2 staining 24 h after the treatments. Our results indicate that the PAMs are neuroprotective in several conditions, including prolonged and acute excitotoxic insults, but not in oxidative stress conditions. Future directions include determining the *in vitro* therapeutic window of the PAMs, and the examination of the PAMs in other *in vitro* models, including oxygen-glucose deprivation and traumatic brain injury (stretch injury), among others. We also aim to profile these PAMs for ADME/ drug-like properties to assess potential neuroprotection in in *in vivo* neuroprotection studies. In conclusion, these novel PAMs of EAAT2 have neuroprotection potential for translation in *in vivo* neurological diseases and conditions.

Disclosures: R. Falcucci: None. **O. Meucci:** None. **J.M. Salvino:** None. **A.C. Fontana:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 502.03/H18

Topic: B.05. Transporters

Title: Lack of interaction between aquaporin-4 (AQP4) and glutamate transporter-1 (GLT1)

Authors: *J. A. HUBBARD, D. K. BINDER;
Ctr. for Glial-Neuronal Interactions, Div. of Biomed. Sci., Univ. of California, Riverside, Riverside, CA

Abstract: Aquaporin-4 (AQP4) and glutamate transporter-1 (GLT1) are astrocyte-specific molecules that regulate water and glutamate homeostasis, respectively. AQP4 modulates bidirectional fluid movement in response to osmotic gradients and GLT1 is responsible for the majority of glutamate uptake in the brain. It has been reported that AQP4 knockout ($AQP4^{-/-}$) mice have reduced levels of GLT1 and, consequently, slowed glutamate uptake (Zeng *et al.*, 2007). This finding has been used to explain altered neurobiological differences in $AQP4^{-/-}$ mice, including increased neuronal activity and impaired long-term potentiation (Szu and Binder, 2016). If GLT1 levels are reduced in $AQP4^{-/-}$ mice, then they may physically interact with one another. We therefore wanted to (1) determine if there is a physical interaction between AQP4 and GLT1 and (2) define GLT1 protein levels in $AQP4^{-/-}$ mice. We used co-immunoprecipitation, Western blot, and immunohistochemistry in both CD1 and C57 wild-type and $AQP4^{-/-}$ mice. We found that GLT1 does not co-immunoprecipitate with AQP4. Furthermore, GLT1 protein levels were not altered in the CD1 or C57 $AQP4^{-/-}$ mice brains compared to their wild-type counterparts. Finally, the immunoreactivity expression patters of GLT1 and AQP4 were drastically different and these two molecules only occasionally co-localized. Therefore, GLT1 and AQP4 likely do not physically interact and a reduction in GLT1 expression cannot explain the altered neurobiology observed in $AQP4^{-/-}$ mice.

Disclosures: J.A. Hubbard: None. **D.K. Binder:** None.

Poster

502. Glutamate Transporters

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Program#/Poster#: 502.04/H19

Topic: B.05. Transporters

Support: FWO

GSKE

VUB

Title: Clinical and central outcomes of peripheral inflammation: what is the role of system xc-?

Authors: *G. ALBERTINI[1], L. DENEYER[1], N. AOURZ[1], E. BENTEA[1], T. DEMUYSER[1], L. VERBRUGGEN[1], H. SATO[2], D. DE BUNDEL[1], A. MASSIE[1], I. SMOLDERS[1];
[1]Vrije Univ. Brussel, Brussel, Belgium; [2]Niigata Univ., Niigata, Japan

Abstract: A variety of pathological stimuli is able to induce systemic inflammation. When systemic inflammation occurs, soluble mediators released from peripheral immune cells are able to reach the central nervous system (CNS) leading to defective brain homeostasis and activation of central immune cells, further exacerbating brain inflammation. Moreover, peripheral inflammation also leads to acute and chronic effects on cognition and behavior. System xc-, with xCT as specific subunit, exchanges intracellular glutamate for extracellular cystine and is the main source of extracellular glutamate in mouse hippocampus and striatum. *In vitro*, it has been shown that several pro-inflammatory stimuli increase the expression of xCT. Besides leading to excessive release of glutamate, increased expression and/or activity of system xc- can directly influence microglia phenotypes towards a pro-inflammatory/neurotoxic state. Modulation of system xc- could therefore be beneficial in inflammation-related disorders in two ways: attenuate the glial release of toxic amounts of glutamate and directly influence microglial polarization towards a protective phenotype. In this study we induced peripheral inflammation via a single injection of 5 mg/kg bacterial lipopolysaccharide (LPS) i.p. and we sought to: i) investigate the consequences of peripheral inflammation on glutamate transporters, ii) unveil the central effects of peripheral inflammation in xCT+/+ and xCT-/- mice and iii) evaluate if deletion of xCT dampens acute clinical implications induced by peripheral inflammation. We demonstrated that LPS strongly impairs glutamate homeostasis *in vivo* leading to significant changes in xCT and GLT1 glutamate transporter protein expression levels in the hippocampus 1 week p.i. Those changes may potentially trigger enhanced hyperexcitability and neuronal death due to toxic extracellular levels of glutamate. We observed that in xCT-/- mice several LPS-induced clinical implications, such as hypothermia, depressive-like behavior in the forced swim and tail suspension tests and increased seizure susceptibility in the pentylenetetrazole model, were

significantly attenuated. Our results highlight the importance of system xc- under inflammatory conditions and bring us one step closer in better understanding its potential relevance in neurological disorders characterized by neuroinflammation.

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Poster

502. Glutamate Transporters

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Program#/Poster#: 502.05/H20

Topic: B.05. Transporters

Support: DIRP NIMH

NIH P41GM103712

Title: Resolution of an anion permeation pathway in glutamate transporters using molecular dynamics simulations and cysteine-scanning analysis.

Authors: ***D. TORRES-SALAZAR**[1], M. H. CHENG[2], A. A. D. GONZALEZ-SUAREZ[1], S. G. AMARA[1], I. BAHAR[2];
[1]Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Excitatory amino acid transporters (EAATs) maintain extracellular glutamate homeostasis in the brain by clearing glutamate through a coupled transport mechanism. They also work as substrate-gated anion channels. In the last decade, structure-function data have advanced our understanding of the substrate transport mechanism. Less is known regarding the structural features responsible for anion permeation. A recent study by Machtens *et al.* combining molecular dynamics (MD) simulations and electrophysiology elegantly showed that channel opening occurs during transitions from intermediate conformations tightly coupled to the transport cycle, an observation that we recently corroborated using different approaches. In addition, they identified a series of residues likely to contribute to the permeation pathway. Here, using molecular modeling and trajectory analysis tools, we visualize the asymmetric transition of substrate-loaded Glt_{Ph}, an archaeal ortholog of the EAATs, into a conformation where one of the subunits assumes an intermediate structure, and we identify a chloride channeling pore intermittently formed at the interface between the trimerization and transport domains of that subunit. The minimum pore size at its constriction zone is around 2.4 Å, close to the value in the

crystal structure of an open chloride channel. The constriction zone is lined by hydrophobic residues (e.g. F50, V51 and L212 in Glt_{Ph}), in broad agreement with those proposed by Machtens *et al*. We have now extended these findings to confirm this permeation pathway in a human isoform (EAAT1) using the substituted cysteine accessibility method. In oocytes expressing several single cysteine substitutions at residues predicted to be within the pathway, we observe an approximately 50% reduction in the macroscopic current amplitude after application of MTSES, a negatively charged sulfhydryl-reactive reagent. We observed comparable results with MTSACE, a similar, but non-charged reagent, ruling out that the observed current reductions were caused by electrostatic interactions. Moreover, our results consistently show that most of the modifications that affect anion permeation do not affect transport currents or glutamate uptake, indicating that substrate translocation is not altered by these modifications. Our results here corroborate a well-defined and evolutionary conserved anion permeation pathway formed mostly by residues in TM2 and TM5. In addition, we demonstrate that despite the well stablished tight coupling between anion channel gating and the transport cycle, anion permeation occurs through a pore independent of the substrate translocation pathway.

Disclosures: D. Torres-Salazar: None. **M.H. Cheng:** None. **A.A.D. Gonzalez-Suarez:** None. **S.G. Amara:** None. **I. Bahar:** None.

Poster

502. Glutamate Transporters

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Topic: B.05. Transporters

Support: DA033436

Title: AAV-mediated upregulation of GLT-1 does not prevent cocaine relapse

Authors: *C. N. LOGAN, L. A. KNACKSTEDT;
Psychology Dept., Univ. of Florida, Gainesville, FL

Abstract: Cocaine addiction is a serious and highly prevalent issue in the United States. Cocaine addiction treatments are complicated by high rates of relapse after treatment. Animal models of relapse such as the operant self-administration extinction reinstatement paradigm can be used to study relapse prevention. In this model the operant response is extinguished and then reinstated using cues previous paired with the cocaine stimulus during self-administration or a dose of the stimulus drug itself. Previous research has shown several neurobiological changes occur in the nucleus accumbens with repeated exposure to cocaine such as the dysregulation of extracellular

nonsynaptic glutamate, downregulation of the cystine-glutamate exchanger and downregulation of GLT-1 transporters. Along with these alterations in the nucleus accumbens, an increase in synaptically released glutamate has been shown to play a role in reinstatement to drug seeking behaviors. The antibiotic ceftriaxone has been shown to upregulate the cystine-glutamate exchanger, upregulate GLT-1 transporters and restore glutamate homeostasis after cocaine use, ultimately preventing reinstatement of drug-seeking behaviors. In efforts to attempt to narrow down which neurobiological alteration from ceftriaxone leads to the prevention of reinstatement of drug seeking behaviors, we used an adeno-associated virus (AAV) to upregulate GLT-1 transporters to investigate whether this would prevent reinstatement of drug seeking. The animals received AAV-GFAP-GLT1 into the nucleus accumbens immediately after the conclusion of two weeks of cocaine self-administration. The animals then underwent three weeks of extinction training before undergoing a cue-primed reinstatement test. In a separate group of animals, prior to drug- and cue-primed reinstatement testing, the animals were probed with microdialysis cannula to enable us to measure glutamate levels. In the presence of AAV-mediated GLT-1 upregulation, animals reinstated cocaine-seeking. These results indicate that ceftriaxone likely induces other protective adaptations in addition to the upregulation of GLT-1 transporters to prevent the reinstatement of cocaine-seeking.

Disclosures: C.N. Logan: None. **L.A. Knackstedt:** None.

Poster

502. Glutamate Transporters

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Program#/Poster#: 502.07/H22

Topic: B.05. Transporters

Support: NSFC31271153

Title: Role of pkc signaling in glioma cells: glutamate transport and metabolism

Authors: *Y. ZHOU[1], R.-Y. LIN[1], Q.-F. LU[1], N. JIA[1], Z.-Q. WANG[1], L. WANG[1], B. R. RANSOM[2], Z. YE[2];
[1]Fujian Med. Univ., Fujian, China; [2]Neurol., Univ. of Washington, Seattle, WA

Abstract: The transport and metabolism of glutamate in glioma cells is surprising flexible. We reported earlier that under proper circumstances, glioma cell glutamate release could reverse direction and become net glutamate uptake, a phenomenon we termed ‘glioma glutamate release reversal’ or GGRR. GGRR has been observed in several human glioma cell lines. The major mechanism of GGRR appears to be dramatic up-regulation of EAAT1 membrane expression that

converts net glutamate release into net glutamate uptake. However, the signaling mechanisms responsible for GGRR remain unknown. We now report that PKC signaling plays a pivotal role in regulating GGRR in a manner significantly different from PKC's role in normal astrocytes. PKC activation by PMA blocks GGRR. Activated PKC eliminated EAAT1 expression, thus preventing GGRR. In addition to decreasing the stability of EAAT1 on glioma cell membranes, PMA treatment inhibited the transcription of EAAT1 mRNA as measured by qPCR. The PMA effect on EAAT1 was completely blocked by the PKC antagonist GF109203X. Besides PMA, other factors activated PKC and negated GGRR. For example, ouabain at nanomolar concentration caused PKC activation and could act synergistically with PMA.

Disclosures: **Y. Zhou:** None. **R. Lin:** None. **Q. Lu:** None. **N. Jia:** None. **Z. Wang:** None. **L. Wang:** None. **B.R. Ransom:** None. **Z. Ye:** None.

Poster

502. Glutamate Transporters

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Topic: B.05. Transporters

Support: NS066019

NS047557

NS075222

MH104318

MH105846

GM097634

EY013079

Title: Conditional deletion of the glutamate transporter GLT-1 in dopaminergic neurons attenuates amphetamine-induced behavioral activation

Authors: ***P. A. ROSENBERG**[1], K. D. FISCHER[1], A. C. W. HOUSTON[1], M. MIAN[1], N. W. HODGSON[1], M. R. DOYLE[2], R. I. DESAI[2], J. BERGMAN[2], A. BECHTHOLT[3], S. CHOI[4], D. L. SULZER[4], E. V. MOSHAROV[4], T. CHOWDHURY[5], A. SHERPA[5], C. AOKI[5];
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Hosp., Belmont, MA; [3]Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD; [4]Neurol., Columbia Univ., New York, NY; [5]New York Univ., New York, NY

Abstract: GLT-1 is the major glutamate transporter in the brain, and is primarily expressed in astrocytes. Although we and others have demonstrated GLT-1 protein expression in excitatory terminals, its function in this location remains unknown. We generated a conditional GLT-1 knockout and inactivated GLT-1 in neurons using synapsin-Cre (synGLT-1 KO). We previously reported [Petr et al., (2015) *J Neurosci.* 35: 5187; Fischer et al., *SfN Abstract* 127.17, (2014); Fischer et al., *SfN Abstract* 386.01, (2015)] that although basal functions, including locomotion, were normal, synGLT-1 KO mice show a blunted locomotor response to acute and chronic administration of amphetamine (AMP). In the present work, we wanted to determine the neuronal type in which deletion of GLT-1 produces a damped response to AMP. Targeted inactivation of GLT-1 specifically from dopamine (DA) neurons using DAT-Cre mimicked the effect of synapsin-cre driven inactivation of GLT-1 on the acute (27% reduction) and sensitized (38% reduction at challenge) locomotor response to AMP. Control experiments showed no effects on responses to AMP of DAT-Cre expression. These observations provide genetic evidence for the expression of GLT-1 in dopamine neurons. Using dual electron microscopy immunocytochemistry (EM-ICC) to detect GLT-1 in axons expressing tyrosine hydroxylase (TH), we found that GLT-1 was co-expressed with TH within DA axons in the NAc shell. Our behavioral and EM results suggested that GLT-1 expressed in DA neurons may promote DA signaling by a vesicular synergy mechanism: GLT-1 might provide the substrate for vGLUT2 mediated transport of glutamate into DA vesicles. However, we found no change in AMP stimulated extracellular DA in the NAc shell by microdialysis, no change in electrically stimulated or AMP-induced DA release in slices using cyclic voltammetry, and no change in DA tissue content. These data suggest that the AMP resistance phenotype in the synGLT-1 KO is unlikely to be due to vesicular synergy or other presynaptic mechanisms. An alternative explanation (see Rimmele et al., poster this meeting) is a change in the expression of proteins involved in DA signaling. Other explanations are possible and will be explored. In summary, genetic and ultrastructural evidence suggests that GLT-1 is expressed in DA neurons, and, in this location plays an important, yet uncharacterized role, in the behavioral response to AMP. Given the significance of AMP as a drug of abuse, and of the AMP sensitized state as a model for the positive features of schizophrenia, it becomes critical to understand the mechanisms underlying the involvement of neuronal GLT-1 in the cellular and circuit effects of AMP.

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Poster

502. Glutamate Transporters

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Topic: B.05. Transporters

Support: Novo Nordisk Fonden NNF14OC0010959

Norwegian Research Council 240844

University of Oslo SERTA

Title: Expression of the EAAT2 glutamate transporter in axon-terminals is more widespread than previously recognized

Authors: *Y. ZHOU, N. DANBOLT;
Inst. of Basic Med. Science, Univ. of Oslo, Oslo, Norway

Abstract: It has been clear since the 1970s that both astrocytes and neurons express glutamate transporters, but the relative contribution of neuronal and glial transporters to the total glutamate uptake activity has been hotly debated ever since. There are five glutamate transporter subtypes (EAAT1-5) in the mammalian CNS. EAAT2 (GLT-1; slc1a2), which is the most important, is predominantly expressed in astroglia although functional EAAT2 protein has been detected in axon-terminals of CA3 hippocampal pyramidal cells and of retinal bipolar cells. However, EAAT2 mRNA is more widespread being detected in most neurons albeit at low levels. Here we investigated whether there is EAAT2 expression in other neuron populations as well. To do this, we generated mice where EAAT2 was selectively deleted in neurons. This was done by crossing our EAAT2-flox mice (B6.Cg-Slc1a2$^{tm1.1Ncd}$/J; Stock no. 026619; Jackson Laboratory; Zhou et al., 2014 J Biol Chem 289:1329-44) with synapsin I-Cre mice (B6.Cg-Tg(Syn1-cre)671Jxm/J; Stock no. 003966; Jackson Laboratory). We then prepared crude synaptosome containing homogenates from various brain regions. Deletion of EAAT2 in neurons did not affect uptake of GABA and did not cause significant reductions in total tissue content of EAAT2 protein as determined by immunoblotting. However, glutamate uptake activity was reduced to about half in synaptosomes from the hippocampus, neocortex, striatum and thalamus. We next tested the efficiency of the synapsin I-Cre driver by crossing these mice with Ai9 reporter mice (B6; 129S6-Gt(ROSA)$^{26Sortm9(CAG-tdTomato)Hze}$/J; Stock no. 007905; Jackson Laboratory). Cells in the latter mice turn red when Cre is expressed. To our surprise Cre had not been expressed in a substantial numbers of neurons. This means that EAAT2 was still present in many of them and that the measured reductions in uptake activities represent underestimations of the true contributions of neuronal EAAT2.

Disclosures: **Y. Zhou:** None. **N. Danbolt:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 502.10/H25

Topic: B.05. Transporters

Support: JSPS

Title: Analysis of effects by repetitive Transcranial Magnetic Stimulation (rTMS) on glutamate transporters, GABA tranporters and glycine transporters on the mouse brain

Authors: ***T. IKEDA**[1,2], N. NUKINA[3,2];
[1]Kochi Univ., Kpchi, Japan; [2]RIKEN, Wake, Japan; [3]Doshisha Univ., Kyoto, Japan

Abstract: rTMS is a noninvasive technique to induce electric current in the brain and is supposed to be beneficial for the treatment of patients with depression, schizophrenia and neurodegenerative disorders. We reported previously that rTMS modulates monoamine tranporter. However, the mechanisms underlying the effects of rTMS are still unclear. We analyzed the changes in mRNA expression in mouse brain that occurred after rTMS with real time PCR. Following 20days of rTMS, many genes were differentially expressed in the mouse brain. Up-regulation of Glutamate transporters (EAAT4, GLAST and GLT1), GABA tranporters (GAT1 and GAT4) and Glycine transporters (GYLT1 and GLYT2) mRNA expression levels were observed. GRP78 (Bip) mRNA expression levels were up-regulated after transient and chronic rTMS. In PC12 cells, an up-regulation of GRP78 (Bip) mRNA and subsequent cell-protective effects were observed after acute and chronic rTMS. These results indicated that the modulation of several genes may be involved in the therapeutic mechanisms of acute and chronic rTMS for patients with neuropsychiatric disorders.

Disclosures: **T. Ikeda:** None. **N. Nukina:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

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Program#/Poster#: 502.11/H26

Topic: B.05. Transporters

Support: NIH grant R01 MH094445-06

CCTST JIT Core grant

Title: Neuronal EAAT2b expression alters excitatory synaptic transmission

Authors: *S. M. O'DONOVAN[1], M. L. BACCEI[2], R. E. MCCULLUMSMITH[1];
[1]Psychiatry and Behavioral Neurosci., [2]Anesthesiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: The excitatory amino acid transporter (EAAT2) is expressed primarily on astrocytes and is responsible for the buffering and transport of over 90% of glutamate from the synapse. Reduced expression of the glutamate transporter EAAT2 is found in several neurocognitive disorders including schizophrenia. EAAT2 localization can significantly impact glutamatergic signaling and transport function. Altered localization of astrocytic EAAT2 shapes excitatory postsynaptic current (EPSC) kinetics and contributes to the regulation of glutamatergic synaptic transmission by controlling the concentration of glutamate at the synapse. Multiple variants of EAAT2 have been identified. EAAT2b has an alternate 11-amino acid long C-terminus which contains a PDZ binding domain. The unique structure of EAAT2b allows for highly regulated localization of the transporter at the synaptic membrane. In schizophrenia, we have found increased expression of the primarily astrocytic EAAT2b in enriched populations of thalamic relay neurons and pyramidal neurons in the anterior cingulate cortex (ACC). We hypothesize that EAAT2b overexpression in pyramidal neurons will increase neuronal glutamate uptake and accelerate decay of EPSCs, diminishing NMDAR activation. These studies will identify the effects of aberrant neuronal EAAT2b expression on glutamatergic signaling within a brain region known to be important for cognitive function, supporting a working model of synaptic dysfunction in chronic schizophrenia. Sprague-Dawley rats will receive an intracerebral injection of AAV1-synapsin-GFP-EAAT2b or control vector AAV1-synapsin-GFP into prelimbic cortex at 7-weeks old. Two weeks after injection, coronal slices (300 μm) will be cut on a vibrating microtome and whole-cell patch clamp recordings of GFP-labelled pyramidal neurons in the prelimbic cortex will be obtained at physiological temperatures. Spontaneous EPSCs (sEPSCs) mediated by AMPARs will first be recorded (from a holding potential of -70 mV) in these EAAT2b-overexpressing pyramidal cells to determine the effect of increased EAAT2b expression on glutamatergic transmission. sEPSC amplitude (pA), frequency (Hz), rise times (ms) and decay times (ms) will be compared between the AAV1-synapsin-GFP-EAAT2b and control groups. In summary, our results will offer insight into the role of enhanced neuronal

EAAT2b in disease states and contribute to our understanding of its effects on glutamatergic synaptic transmission, simulating changes observed in schizophrenia in an effort to understand the consequences of aberrant localization of this glutamate transporter splice variant in the brain.

Disclosures: S.M. O'Donovan: None. **M.L. Baccei:** None. **R.E. McCullumsmith:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

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Program#/Poster#: 502.12/I1

Topic: B.05. Transporters

Support: NIH NS066019

NIH MH104318

NIH HD018655

Tommy Fuss Fund

T32 NS007473

Title: Biochemical phenotyping of the neuronal GLT-1 knockout mouse

Authors: *T. S. RIMMELE[1], K. D. FISCHER[1], R. B. LAPRAIRIE[2], E. M. DENOVAN-WRIGHT[2], P. A. ROSENBERG[1];
[1]Dept. of Neurol. and the F.M. Kirby Neurobio. Ctr., Boston Children's Hosp., Boston, MA;
[2]Dept. of Pharmacol., Dalhousie Univ., Halifax, NS, Canada

Abstract: To reveal cell-type-specific functional roles of the brain's major glutamate transporter, GLT-1, we generated a conditional GLT-1 knockout mouse with which to inactivate GLT-1 in neurons using synapsin-Cre (synGLT-1 KO) and in astrocytes using inducible human GFAP-CreERT2 [Petr et al., (2015) *J Neurosci.* 35: 5187]. We found that GLT-1 in astrocytes protects against fatal epilepsy, whereas the loss of neuronal GLT-1 significantly diminishes glutamate uptake into synaptosomes. As previously reported [Fischer et al., *SfN Abstract* 127.17, (2014); Fischer et al., *SfN Abstract* 386.01, (2015)], we sought to understand the functional significance of GLT-1 expression in neurons and discovered that pan-neuronal GLT-1 knockout resulted in a phenotype of amphetamine (AMP) resistance. We used Cre-drivers to determine whether this phenotype could be associated with a specific population of neurons, and found that dopamine transporter (DAT)-Cre driven deletion of GLT-1 specifically in dopamine (DA) neurons

replicated the phenotype of pan-neuronal knockout of GLT-1 (see poster this meeting). In that study, we found no evidence for a change in DA dynamics, arguing against an effect on vesicular synergy or other presynaptic mechanisms. Another possibility we considered was that there might be a constitutive change in expression of genes involved in DA signaling in the synGLT-1 KO causing a change in response to AMP. Using gene microarray to assay changes in gene expression in the striatum, we found no changes of expression attained at a significance level of $p<0.01$; however, there was a suggestion of a decrease in D1 receptor expression and DARPP-32 (Ppp1r1b) in the synGLT-1 KO mice at the $p<0.05$ level. Using *in situ* hybridization we detected decreased D2 receptor expression in the prefrontal cortex of 12 week old synGLT-1 KO mice compared to WT (12.2 ± 1.2 in WT, 3.2 ± 0.9 in synGLT-1 KO, $p<0.01$), but no changes in other brain areas or in D1 receptor expression. No change in D2 receptor expression was found in 6 week old synGLT-1 KO mice. In summary, we have obtained evidence that postsynaptic changes in DA receptors, in particular in D2 receptors, occur in the synGLT-1 KO. This downregulation of D2 receptors might contribute to the phenotype of AMP resistance seen in this mutant. Other possible mechanisms underlying the observed phenotype include mGluR modulation of DA release or reduced glutamate signaling by DA neurons.

Disclosures: T.S. Rimmele: None. **K.D. Fischer:** None. **R.B. Laprairie:** None. **E.M. Denovan-Wright:** None. **P.A. Rosenberg:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

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Program#/Poster#: 502.13/I2

Topic: B.05. Transporters

Support: Department of Veterans Affairs Merit Review Program

Queen Elisabeth Medical Foundation

FWO

VUB - SRP

Title: Pre- and postsynaptic changes at excitatory synapses in xCT deficient mice

Authors: *A. MASSIE[1], E. BENTEA[1], C. MOORE[2], M. J. CHURCHILL[2], R. L. HOOD[2], L. DENEYER[1], L. VERBRUGGEN[1], H. SATO[3], C. K. MESHUL[4];
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Abstract: System xc- is a plasma membrane amino acid antiporter, of mainly glial origin, that couples the import of cystine with the export of glutamate. System xc- (specific subunit xCT) contributes substantially to ambient extracellular glutamate levels in various regions of the brain, including the striatum and hippocampus. Despite the fact that system xc- is highly expressed in the brain and is a proposed therapeutic target for various neurological disorders, including Parkinson's disease, Alzheimer's disease, multiple sclerosis and epilepsy, its function under physiological conditions in the central nervous system remains poorly understood. By acting as a source of glial extrasynaptic glutamate, system xc- might modulate synaptic transmission as a mechanism of neuro-glial communication. Previous electrophysiological findings indicate that system xc- delivered glutamate can inhibit excitatory synaptic neurotransmission in the cortico-accumbens pathway (Moran et al. J Neurosci. 2005; 25:6389-93) and at hippocampal CA3-CA1 synapses (Williams et al. J Neurosci. 2014; 34:16093-102). In order to gain further insight into the proposed function of system xc- as modulator of synaptic transmission, we carried out single section electron microscopy analyses of excitatory axospinous synapses at the level of the dorsolateral striatum and motor cortex of adult xCT knockout (xCT-/-) and xCT wildtype (xCT+/+) mice. Our findings accommodate the hypothesis that system xc- negatively modulates neurotransmission, as morphological changes in the excitatory synapses in the dorsolateral striatum of xCT-/- mice reflect increased synaptic activity. In particular, we could observe depletion of glutamate immunogold labeling from presynaptic terminals of xCT-/- mice, an increase in the head diameter and area of spines contacted by asymmetric synapses, an increase in the length, thickness and area of the postsynaptic density, an increased occurrence of spinules, and an increase in the average area of synaptic vesicles. The ultrastructural changes observed in xCT deficient mice suggest the involvement of both presynaptic and postsynaptic forms of synaptic strength regulation via system xc-. In the future we would like to extend our findings on excitatory synapses in the motor cortex, as well as evaluate the expression of AMPA and NMDA receptor expression as a possible contributor to the increased size of the postsynaptic density in xCT deficient mice. Together, these findings shed new light on the re-organization of the glutamatergic system after genetic deletion of system xc-, and confirm the involvement of this antiporter in the control of synaptic strength *in vivo*.

Disclosures: **A. Massie:** None. **E. Bentea:** None. **C. Moore:** None. **M.J. Churchill:** None. **R.L. Hood:** None. **L. Deneyer:** None. **L. Verbruggen:** None. **H. Sato:** None. **C.K. Meshul:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 502.14/I3

Topic: B.05. Transporters

Support: Intramural Research Program of the National Institute of Mental Health

Title: The trace amine receptor 1 and amphetamine induced internalization of the dopamine and glutamate transporters

Authors: *S. M. UNDERHILL, P. HULLIHEN, J. CHEN, S. AMARA;
Lab. of Mol. and Cell. Neurobio., Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Amphetamine (AMPH) and its derivatives are useful therapeutic agents, but also pose a danger as a consequence of their addictive properties. Acute AMPH exposure elevates extracellular dopamine by a variety of mechanisms including an increase in the rate of internalization of the plasma membrane dopamine transporter (DAT) and of the excitatory amino acid transporter 3 (EAAT3). We found that AMPH-mediated trafficking requires activation of the small GTPase RhoA that is regulated by protein kinase A (PKA) phosphorylation. PKA phosphorylation of RhoA takes it out of the active pool and, effectively stops activation of the GTPase as well as neurotransmitter transporter internalization. The trace amine receptor 1 (TA1) is a G-protein coupled receptor (GPCR) known to couple through Gs and activate PKA signaling. TA1 also has been shown previously to contribute to the actions of psychostimulants in dopamine neurons and thus these observations led us to examine the potential role of TA1 in RhoA and PKA activation.

We used CRISPR-Cas9 gene editing technology to disrupt the TA1 gene in HEK293 cells. We hypothesized that the actions of AMPH on transporter trafficking in TA1 knockout cells would be potentiated because without TA1- mediated PKA signaling the activation of RhoA would be sustained. Unexpectedly, in cells that lack TA1, AMPH did not induce DAT or EAAT3 internalization. We also could not detect Rho activation despite robust AMPH-induced Rho activation in wildtype TA1-expressing cultures. AMPH-induced Rho activation and DAT/EAAT3 internalization could also be restored in the knockout cell line by transfecting with a modified TA1. TA1 was initially characterized as a Gs-coupled GPCR, however these data suggest that alpha-subunits that couple to other signaling pathways can interact with the receptor. Using mini-genes that interfere with various alpha-subunits we determined that TA1 also couples with the alpha-13 G-protein, well-established as an activator of RhoA signaling.

These observations show that TA1 serves as the intracellular target that mediates the effects of AMPH on RhoA and cAMP signaling and suggest new pathways to target in order to better understand the mechanisms of action of AMPH.

Disclosures: **S.M. Underhill:** None. **P. Hullihen:** None. **J. Chen:** None. **S. Amara:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 502.15/I4

Topic: B.05. Transporters

Title: Habit learning induced by natural reward involves molecular adaptations of the glutamate transporter 1 in the dorsolateral striatum

Authors: *A. J. BOENDER, R. TONINI;
Neurosci. and brain technologies, Inst. Italiano di Tecnologia, Genova, Italy

Abstract: Uncovering the mechanistic similarities and differences between drug-induced habits and naturally induced habits should explain why these two types of habit acquisition differ in their ability to be reversed. Habitual drug seeking is associated with downregulation of the glutamate transporter 1 (GLT1) and xCT, the catalytic subunit of the constitutive cysteine/glutamate exchanger, which leads to imbalances in the glutamate homeostasis. This results in decreased extrasynaptic glutamate levels and a reduced inhibitory tone on corticostriatal synapses via metabotropic glutamate receptors, ultimately increasing the release probability of synaptic glutamate. Whether xCT and GLT1 are also involved in the formation of habits that occurs naturally after repeated practice remains to be established. To address this question, mice were overtrained to nose poke for food rewards, a form of operant conditioning that promotes habitual behavior. Upon habit formation, xCT mRNA levels were unaffected, whilst levels of GLT1 mRNA and protein were upregulated in the dorsolateral striatum (DLS); a brain region critical for the expression of habitual behavior. Since GLT1 protein is predominantly expressed by astrocytes, this points toward astrocytic involvement in habit learning. The apparent discrepancy between the downregulation of GLT1 by drug-induced habits and the upregulation of GLT1 upon the acquisition of a habit induced by food rewards could be explained by the differential regulation of GLT1 isoforms, for which we have found proof using PCR-based techniques. In sum, we provide evidence that habit learning by repeated practice affects the glutamate homeostasis in the DLS through increased GLT1 expression.

Disclosures: **A.J. Boender:** None. **R. Tonini:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 502.16/I5

Topic: B.05. Transporters

Support: NS051445-06

NS082982

Title: System x_c^- null mice are resistant to pentylenetetrazole kindling.

Authors: *S. M. SHAHIDZADEH, J. A. HEWETT, S. J. HEWETT;
Syracuse Univ., Syracuse, NY

Abstract: Kindling, the time-dependent sensitization of neuronal circuitry to an electrical or chemical stimulus, serves as a model of epileptogenesis. This neuroplasticity is associated with aberrant activation of the glutamatergic system. System x_c^- is an antiporter known to contribute to basal extracellular glutamate levels. Thus, in this study, we tested the contribution of system x_c^- to epileptogenesis using a chemical kindling model. Kindling in male and female wild-type or system x_c^- null littermates was induced by giving a subconvulsant dose of pentylenetetrazole (PTZ) once daily (i.p.) for 21 days. Animals were observed for 30 min and seizure activity scored according to a slightly modified Racine scale. Animals were considered kindled after exhibiting a convulsive seizure on three consecutive days. Our data demonstrate the incidence of kindling was significantly decreased in system x_c^- deficient mice as compared to wild-type littermates. Specifically, in response to a daily injection of 35 mg/kg PTZ, 74% (17/23) of wild-type male mice kindled, as compared to only 43% (6/14) of their system x_c^- null male littermates. A similar result was found in females. When subjected to a daily injection of 28 mg/kg PTZ, the incidence of kindling in wild-type female mice was 31% (4/13), whereas it was only 14% (1/7) in system x_c^- null female littermates. Furthermore, persistence of the kindled state was decreased in mice lacking system x_c^- compared to their wild-type littermate controls. To wit, 81% (17/21) of wild-type and only 57% (4/7) of system x_c^- null mice exhibited a convulsive seizure when re-challenged with PTZ 10 days post-kindling. Altogether, these data suggest that system x_c^- contributes to the enhancement of excitatory transmission that is responsible for the kindled phenotype, thereby suggesting that system x_c^- may play a permissive role in epileptogenesis. Supported by NS051445-06 (SJH and JAH) and NS082982 (JAH).

Disclosures: **S.M. Shahidzadeh:** None. **J.A. Hewett:** None. **S.J. Hewett:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 502.17/I6

Topic: B.05. Transporters

Support: Ruth D. and Ken M. Davee Pediatric Neurocritical Care Program

Title: Thrombin decreases expression of GLT-1 via the rho kinase pathway in astrocytes

Authors: *A. HOLLOWAY[1], C.-S. PIAO[2], M. WAINWRIGHT[3];
[1]Neurol., Ann and Robert H Lurie Children's Hosp., Chicago, IL; [2]Neurol., [3]Pediatrics, Neurology, Critical Care, Ann and Robert H Lurie Children's Hosp. of Chicago, Chicago, IL

Abstract: Background: Traumatic brain injury (TBI) can cause decrease in the expression of glutamate transporters (EAAT1/GLAST and EAAT2/GLT-1), which may contribute to glutamate excitotoxicity and secondary brain injury. TBI also causes compromise of the blood brain barrier and extravasation of macromolecules, such as thrombin, into the brain parenchyma. The mechanism by which TBI leads to a reduction in expression of astrocyte glutamate transporters is not known. The Rho/Rho kinase (ROCK) pathway has previously been implicated as a mediator of glutamate transporter expression. Here, we used an in vitro model of TBI to test the hypothesis that mild mechanical stretch injury would exacerbate subsequent thrombin exposure, causing decreased expression and impaired function of GLAST and GLT-1 in hippocampal astrocytes via the ROCK pathway. Methods: Primary hippocampal astrocytes were isolated from Sprague Dawley rat pups and plated on flexible-bottom cell culture plates. Astrocytes were subjected to a mild stretch injury and exposed to thrombin or vehicle one hour later. To elucidate the role of ROCK in regulation of glutamate transporters, we tested the effect of the ROCK inhibitors, Y-27632 and Fasudil, on glutamate transporter expression and function under the same conditions. Cells were assessed for expression of glutamate transporters and activation of the Rho kinase pathway by Western Blot, or were subjected to a glutamate uptake assay. Media samples were evaluated for lactate dehydrogenase (LDH) content to confirm injury. Results: Exposure to thrombin or stretch injury alone caused down-regulation of GLAST and GLT-1. Exposure to thrombin after stretch injury caused significantly greater down-regulation of both transporters compared to control cells and stretch injury alone. Glutamate uptake was also significantly decreased at 24 hr recovery following exposure to thrombin, consistent with the down-regulation of both transporters. Stretch injury did not alter glutamate uptake and there was no additive effect of stretch injury when combined with exposure to thrombin. Exposure to thrombin activated the ROCK pathway and inhibition of ROCK activation reversed the thrombin-induced down-regulation of GLT-1. Conclusion: These results suggest that thrombin contributes to the regulation of the expression of astrocyte glutamate transporters and glutamate

uptake, acting via the ROCK pathway and that these responses are altered following stretch injury during TBI.

Disclosures: **A. Holloway:** None. **C. Piao:** None. **M. Wainwright:** None.

Poster

502. Glutamate Transporters

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 502.18/I7

Topic: B.12. Glial Mechanisms

Support: OGMF Conacyt 261310

AO Conacyt 79502

AO-MN Conacyt-FNRS 210238

Title: Glutamate-dependent glut3-glast association in cultured bergmann glia cells

Authors: O. G. MENDEZ-FLORES[1], *L. C. HERNANDEZ[2], E. SUAREZ-POZOS[1], M. NAJIMI[3], A. ORTEGA[1];
[1]Toxicology, [2]Ctr. De Investigacion Y Estudios Avanzados Del IPN, Mexico City, Mexico; [3]Inst. de Recherche Expérimentale et Clinique, Univ. Catholique de Louvain, Brussels, Belgium

Abstract: Glutamate (Glu), the main excitatory neurotransmitter in the vertebrate brain, exerts its actions through specific membrane receptors present in neurons and glial cells. Over-stimulation of glutamate receptors results in neuronal death, unless glial sodium-dependent glutamate uptake transporters, remove the amino acid from the synaptic cleft.
The sustained Na^+ influx associated to Glu uptake in glial cells, activates the Na^+/K^+ ATPase to restore the ionic balance, additionally Glu entrance activates glutamine synthetase. Both biochemical processes are energy demanding and therefore, activate glucose uptake. In order to gain insights into the molecular mechanisms underlying the Glu and glucose uptake interaction we performed [^{3}H]-2-deoxy-D-glucose ([^{3}H]2DOG) uptake assays, immunoprecipitation, biotinylation and western blot in the well-known model of Bergmann Glia cell primary (BGC) culture.
Glu stimulation, presumably through Glu transporters, as indicated by the ability of D-Asp to increase [^{3}H]2DOG uptake, augments global glucose transporters expression, as well as their plasma membrane trafficking. Additionally a physical interaction between GLUT3 and GLAST was noted upon Glu and D-Asp treatment. The present study shows a rapid response of glial cells

to Glu neurotransmitter exposure, which entails a likely dialogue between neuronal and glial cell population in the vicinity of glutamatergic synapses.

Disclosures: O.G. Mendez-Flores: None. **L.C. Hernandez:** None. **E. Suarez-Pozos:** None. **M. Najimi:** None. **A. Ortega:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 503.01/I8

Topic: B.06. Neurotransmitter Release

Support: NARSAD Young Investigator Award

Spanish Ministry of Education and Science (BFU2015-63769-R)

Junta de Comunidades de Castilla-La Mancha (PPII-2014-005-P)

Title: Phogrin, a novel vesicle marker in the CNS, is expressed in hippocampal parvalbumin-positive interneurons

Authors: *S. JURADO[1], R. LUJAN[2], F. MUNOZ-CUEVAS[1], J. RAMIREZ-FRANCO[1]; [1]Univ. of Maryland, Baltimore, MD; [2]Synaptic Structure Laboratory, Inst. de Investigación en Discapacidades Neurológicas (IDINE), Univ. de Castilla La Mancha, Albacete, Spain

Abstract: Hippocampal interneurons comprise a diverse family of inhibitory neurons which function is critical for fine information processing. Along with gamma-aminobutyric acid (GABA), interneurons secrete a myriad of neuroactive substances via secretory vesicles which molecular composition and regulatory mechanisms remain unknown. In this study, we have combined immunohistofluorescence, electron microscopy and transgenic mice to describe the molecular content of vesicles in distinct populations of hippocampal neurons. Our results indicate that chromogranin B, a canonical large dense core vesicle marker, is excluded from inhibitory cells in the hippocampus but highly expressed in excitatory CA3 pyramidal neurons and dentate gyrus granule cells. Surprisingly, phogrin, an integral protein of secretory vesicles in neuroendocrine cells, is highly enriched in parvalbumin-positive interneurons. Consistently, immunoelectron microscopy revealed phogrin staining in axon terminals of symmetrical synapses establishing inhibitory contacts with cell bodies of CA1 pyramidal neurons. Furthermore, phogrin is highly expressed in CA3 and dentate gyrus interneurons that also contain neuropeptide Y. Our results provide the first evidence of phogrin expression in

hippocampal interneurons and suggest the existence of molecularly distinct populations of secretory vesicles in different neuronal types.

Disclosures: S. Jurado: None. **R. Lujan:** None. **F. Munoz-Cuevas:** None. **J. Ramirez-Franco:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 503.02/I9

Topic: B.06. Neurotransmitter Release

Support: CIHR Fellowship

NHMRC Project Grant

Title: Isolating the bulk endosome from nerve terminals

Authors: *L. C. MILLER[1], L. GATTO[2,3], P. HAINS[1], E. KETTLE[1,4], L. BRECKELS[2,3], R. A. BOADLE[4], K. LILLEY[3], P. J. ROBINSON[1];
[1]Children's Med. Res. Inst., Westmead, Australia; [2]Computat. Proteomics Unit, Dept. of Biochem., [3]Cambridge Ctr. for Proteomics, Dept. of Biochem., Univ. of Cambridge, Cambridge, United Kingdom; [4]Electron Microscope Res. Facility, The Westmead Inst. for Med. Res., The Univ. of Sydney, Westmead, Australia

Abstract: Bulk endocytosis is the internalization of large amounts of membrane observed in various cell types. In neuronal synapses activity dependent bulk endocytosis (ADBE) occurs during high activity to compensate for large amounts of internal membrane that fuse with the plasma membrane. ADBE is more rapid than constitutive clathrin mediated endocytosis and is triggered by high activity. New synaptic vesicles (SVs) derived from the bulk endosomes primarily refill the recyclable pool of vesicles yet the intervening steps from BEs to SVs are unknown. Our aim is to identify the proteins localised to BEs as the first step towards understanding ADBE regulation. To achieve this we developed a purification method to isolate BEs from SVs for subsequent mass spectrometry (MS) analysis. Methods: Percoll synaptosomes obtained from whole rat brains were treated with control or high K^+ stimulation, at a level known to predominantly induce BE formation, in the presence of HRP as a fluid phase uptake marker. The synaptosomes were lysed and organelles separated on a density gradient. The protein content of each fraction was isolated on beads and digested for MS analysis, or processed for Western blots (WB). Digested peptides from control and stimulated fractions were differentially labelled

by dimethylation and analysed by LC-MS on an ABSciex Triple TOF 5600. Digested peptides from select density fractions of control treated synaptosomes were labelled using 10-plex TMT and analysed by LC-MS on a Thermo Q Exactive. Organelles were collected from combined density fractions of synaptosomes stimulated in the presence of HRP. The presence of HRP in the organelles was detected by electron microscopy (EM) using DAB/H_2O_2. Results/Conclusion: Analysis of HRP and overall protein changes down the gradient indicate 3 distinct regions of newly endocytosed organelles suggesting 2 populations of BEs. Localisation of organelle proteins by isotope tagging (LOPIT) analysis of the TMT data dissociated the continuous gradient of proteins into distinct organelle clusters. Tracking proteins moving into the putative BE regions and relating them to the LOPIT analysis reveals the organelles likely involved in BE formation. EM images confirm the presence of large HRP labelled structures in a putative BE region.

Disclosures: **L.C. Miller:** None. **L. Gatto:** None. **P. Hains:** None. **E. Kettle:** None. **L. Breckels:** None. **R.A. Boadle:** None. **K. Lilley:** None. **P.J. Robinson:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Support: JHU startup fund

HHMI

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SFB 665

SFB 958

Title: Molecular mechanisms of ultrafast endocytosis

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Abstract: Synaptic vesicles are recycled locally at synapses. Using 'flash-and-freeze' time-resolved electron microscopy, we have discovered a novel endocytic pathway, ultrafast endocytosis, that retrieves vesicle membrane within 100 ms after vesicle fusion. Despite its fast kinetics, this pathway requires dynamin for the fission reaction. However, little is known about the molecular mechanisms that mediate maturation of endocytic pits from the planar membrane. Here, we have used a candidate approach to test the involvement of molecules that directly or indirectly interact with dynamin in this process. Endophilin is a membrane-bending protein that interacts with dynamin directly through its Src homology 3 (SH3) domain. Genetic knockout of endophilin A results in an accumulation of clathrin-coated vesicles in the synaptic terminals. Following a single stimulus, ultrafast endocytosis is triggered in these neurons, but endocytic structures are stalled on the membrane due to the delayed formation of the neck. These observations indicate that endophilin is likely required at a late step of ultrafast endocytosis rather than for the initiation of membrane curvature. Synaptojanin is a phosphoinositide phosphatase that interacts with endophilin through its proline-rich domain. It contains two phosphatase domains: Sac1 like phosphatase and inositol 5-phosphatase. Synaptojanin and endophilin function together during clathrin-mediated endocytosis. To test its role in ultrafast endocytosis, we stimulated mouse hippocampal neurons lacking synaptojanin 1. Similar to the endophilin knockout neurons, endocytic structures are stalled on the membrane with a significant delay in the neck formation. This phenotype is rescued by expressing full-length synaptojanin in these neurons. However, when 5-phosphatase activity, but not the Sac1 activity, is blocked by a point mutation, the neck formation is delayed, suggesting that the removal of a phosphate group from the 5-postion of inositol is required for shape transition during ultrafast endocytosis. Taken together, these results suggest that endophilin and synaptojanin mediate the neck formation during ultrafast endocytosis.

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Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 503.04/I11

Topic: B.06. Neurotransmitter Release

Support: KRIBB Research Initiative Program (Korean Biomedical Scientist Fellowship Program), Korea Research Institute of Bioscience and Biotechnology, Republic of Korea

Title: Clathrin is essential for vesicle endocytosis at mammalian central synapses

Authors: *S. LEE, S. VILLARREAL, X.-S. WU, H. LIU, Y. JIN, L.-G. WU;
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Abstract: Synaptic vesicle endocytosis retrieves fused vesicles from the plasma membrane to sustain synaptic transmission crucial for brain function. It has been hypothesized to be mediated primarily by the classical clathrin-dependent mechanism for decades. However, recent test of clathrin hypothesis with manipulation of clathrin and electron microscopy suggests surprisingly that clathrin is dispensable for synaptic vesicle endocytosis at mammalian central synapses. Here we determined whether clathrin is required for synaptic vesicle endocytosis by performing a conditional knockout of clathrin heavy chain and pHluorin imaging. We found that endocytosis of synaptic vesicle protein synaptophysin and VAMP2 at cultured mouse hippocampal synapses is slow and clathrin-dependent after 1-800 action potentials at 0.03-80 Hz. Also, these results suggest that clathrin is essential for synaptic vesicle endocytosis in hippocampal synapses.

Disclosures: **S. Lee:** A. Employment/Salary (full or part-time): Fellowship from Korea Research Institute of Bioscience and Biotechnology. **S. Villarreal:** None. **X. Wu:** None. **H. Liu:** None. **Y. Jin:** None. **L. Wu:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Topic: B.06. Neurotransmitter Release

Support: NIH Grant R01NS082759

Title: Activity- and temperature-dependent facilitation of membrane endocytosis at nerve terminals by a non-canonical mechanism

Authors: H.-Y. YUE, *J. XU;
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Abstract: In nerve terminals, endocytosis follows immediately after exocytosis to recover membrane homeostasis of terminals and recycle vesicle membrane for future exocytosis. Endocytosis typically retrieves membrane by an amount equivalent to the exocytosed, while

membrane capacitance from various cells has revealed excess endocytosis retrieving extra membrane that pre-exists at surface. To advance understanding of endocytosis of pre-existing membrane, we performed membrane capacitance measurements at the rat calyx of Held. We found that endocytosis of pre-existing membrane was ATP-independent, distinguishing it from the well-recognized clathrin-mediated endocytosis, bulk endocytosis and ultrafast endocytosis. This non-canonical form of endocytosis played a minor role and required intense activity to trigger at room temperature, but became significant at near physiology temperature. These results suggest that endocytosis of pre-existing membrane is a unique, important mechanism to ensure efficiency of endocytosis.

Disclosures: H. Yue: None. **J. Xu:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Topic: B.06. Neurotransmitter Release

Support: NIH Grant NS080946

Alzheimer's Association and Global Down Syndrome Foundation

Title: Synaptojanin phosphorylation by the minibrain/DYRK1A kinase regulates synaptic vesicle endocytosis

Authors: L. WANG[1], J. GENG[1], J. LEE[2], C.-K. CHEN[1], *K. T. CHANG[3];
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Abstract: The rapid recycling of synaptic vesicles during neuronal activity is critical for maintaining communication between neurons. Defects in synaptic vesicle recycling can therefore have severe consequences, ranging from neurodegeneration to lethality. Synaptojanin (Synj), a phosphoinositide phosphatase, is known to play an important role in synaptic vesicle endocytosis by facilitating the uncoating of clathrin following synaptic vesicle uptake. Previous work from our lab demonstrated that Synj is a substrate of the minibrain (Mnb) kinase, a homolog of human dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) in *Drosophila*; however, the target sequence on Synj phosphorylated by Mnb has not been identified and the functional impacts of Synj phosphorylation by Mnb are not well understood. We find that Ser1029 is a target site on Synj that is phosphorylated by Mnb *in vivo*. In addition,

phosphorylation of Synj at Ser1029 is required to maintain normal synaptic vesicle recycling *in vivo* but surprisingly is not necessary to sustain neurotransmission during high frequency stimulation. Additional experiments will be performed to distinguish mechanisms underlying synaptic vesicle recycling by phosphorylated and dephosphorylated Synj. Since both Mnb and Synj are upregulated in DS, and Synj is mutated in Parkinson's disease, an understanding of mechanisms that modulate Synj function will provide valuable insights into basic mechanisms affecting neuronal communication and lead to strategies to treat neurological disorders.

Disclosures: **L. Wang:** None. **J. Geng:** None. **J. Lee:** None. **C. Chen:** None. **K.T. Chang:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Topic: B.06. Neurotransmitter Release

Support: NIH Grant RO1GM111997

AHA Grant 13SDG14420049

Title: A supported tubulated bilayer system for evaluating synaptotagmin effects on membrane curvature

Authors: ***P. DAHL**[1], J. VASQUEZ[2], J. KNIGHT[2], A. ANANTHARAM[1];
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Abstract: The proper execution of Ca^{2+}-triggered exocytosis requires extreme changes in bilayer shape to be precisely regulated. Because membranes alone are unlikely to undergo the required changes, proteins are necessary to mediate events. However, the identity of these proteins and the conditions under which they act are not well understood. Within this context, our studies focus on membrane targeting C2 domains from synaptotagmin-7 (Syt-7) – an isoform of the Syt protein family that is important for secretion in neuroendocrine cells. To define how Syt-7 drives changes in membrane morphology, we have used recombinant C2AB protein fragments, supported lipid bilayers (SLBs), and total internal reflection fluorescence microscopy (TIRFM). We have developed conditions for forming SLBs under which the membrane spontaneously forms long tubule-like structures extending away from the support surface. Using this Supported Tubulated Bilayer System (STuBS), we find that addition of purified Syt-7 C2AB, but not Syt-1 C2A, leads to disappearance of the tubules in a Ca^{2+} dependent manner; the mechanism of this

dramatic change is currently under investigation. These studies demonstrate that Syt-7 can alter membrane morphology, ostensibly by driving changes in membrane curvature. They also demonstrate the utility of STuBS as a novel experimental system in which protein-mediated changes in membrane topology can be studied in aqueous media and in real-time.

Disclosures: P. Dahl: None. **J. Vasquez:** None. **J. Knight:** None. **A. Anantharam:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Program#/Poster#: 503.08/J3

Topic: B.06. Neurotransmitter Release

Title: Two major synaptotagmin isoforms cooperate to drive release at a fast inhibitory synapse

Authors: *B. BOUHOURS, O. KOCHUBEY, E. GJONI, R. SCHNEGGENBURGER; EPFL, Lausanne, Switzerland

Abstract: Fast transmitter release at synapses is enabled by the C2-domain protein Synaptotagmin (Syt). Syt1 is the major Ca^{2+} sensor for fast release at excitatory forebrain synapses whereas Syt2, a Syt isoform with high sequence homology to Syt1, is the Ca^{2+} sensor for phasic release at excitatory hindbrain synapses. The role of Syt isoforms at inhibitory synapses is, however, less well known. Although inhibitory synapses in cortical neuron cultures were shown to use Syt1 (Maximov and Südhof, 2005), the calcium sensor at defined inhibitory synapses of more intact preparations has remained elusive, with only a minor role for Syt1 at GABAergic output synapses of fast-spiking hippocampal interneurons (Kerr et al., 2008).
Here we studied the role of the two major Syt isoforms, Syt1 and Syt2, at a strong and fast-releasing inhibitory synapse in the auditory brainstem, the MNTB to LSO synapse. To overcome the perinatal lethality of the conventional Syt1 KO mice, we used a novel floxed allele of Syt1, bred together with a conventional Syt2 KO mouse. To produce either single- or double KO inhibitory synapses amenable to optogenetic stimulation, we virally expressed Cre-recombinase and the Channelrhodopsin variant oChIEF in presynaptic neurons of the different genotype combinations. This method allowed us to limit synaptic stimulation to molecularly perturbed presynaptic elements.
We found that both Syt1- and Syt2 must be deleted to produce strongly asynchronous release typical for Syt KO synapses. In contrast, single KO synapses had only small deficits in phasic release, but we observed an increased spontaneous release in Syt2 KO synapses. The redundant action of two major Syt isoforms might guarantee high rates of fast release at these inhibitory

synapses, a mechanism with possible relevance also for fast-releasing inhibitory synapses in other brain areas.

Disclosures: B. Bouhours: None. **O. Kochubey:** None. **E. Gjoni:** None. **R. Schneggenburger:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Topic: B.06. Neurotransmitter Release

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AHA Grant (13SDG14420049)

Title: Chromaffin cell synaptotagmin isoforms form functionally but not spatially separable granule pools

Authors: *T. C. RAO[1], M. W. SCHMIDTKE[1], E. R. CHAPMAN[2], D. R. GIOVANNUCCI[3], A. ANANTHARAM[1];
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Abstract: Current models for the adrenal chromaffin cell secretory response rely on the assumption that secretory granules are functionally homogenous. However, there is growing evidence that heterogeneity may define fundamental properties of granules, including their kinetics and Ca^{2+}-dependence of fusion. In this context, a key difference between granules appears to be whether they express synaptotagmin-1 (Syt-1) or synaptotagmin-7 (Syt-7). To understand the implications of this difference for the secretory response, we first investigated the morphological distribution of Syt-bearing granules within cells. Despite the fact that isoforms are rarely co-expressed (<10%), Syt-1 and Syt-7-bearing granules are homogenously distributed within cells and lack obvious evidence of spatial segregation with respect to the plasma membrane or distinct coupling to Ca^{2+} channel subtypes. We next investigated functional differences between granules with Syt-1 or Syt-7 in cells depolarized with elevated K^{+}. First, we found that with mild depolarization, the Syt-7 granule population shows far greater fusion efficacy. However, strong depolarization is equally effective at driving fusion of both types of Syt-bearing granules. When cells are challenged to secrete again immediately following a strong depolarization, Syt-1 granules require less time to transition to a “fusion-ready” state.

Irrespective of the fusion efficacy, Syt-7 granules fuse with faster kinetics across all stimulation conditions tested. These findings were validated in cells permeabilized with digitonin and exposed directly to high intracellular Ca^{2+}. While Syt-7 granules fuse with greater efficacy at low $[Ca^{2+}]_{in}$, higher $[Ca^{2+}]_{in}$ is equally effective at activating isoforms. Overall, our results show that Syt-1 and Syt-7 are sorted to functionally, but not spatially, distinguishable granule pools. We posit that the heterogeneity in chromaffin granule fusion modes and release kinetics may therefore arise from intrinsic differences in granules themselves.

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Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Topic: B.06. Neurotransmitter Release

Support: MOST 103-2311-B-002-026-MY3

Title: Calcium binding to Synaptotagmin III regulates the kinetics of fusion pores and the interaction with SNAP-25

Authors: Y.-H. TSAI[1], Y.-T. HUANG[1], *C.-T. WANG[1,2,3,4];
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Abstract: Calcium-dependent exocytosis is initiated by calcium binding to a calcium sensor protein, triggering a number of interactions with molecular targets, including the fusion machinery SNAREs. Synaptotagmins (Syts) function as calcium sensors that regulate the kinetics of exocytosis in neurons and neuroendocrine cells. At least 17 Syt isoforms have been found in mammals and share a similar structure, consisting of an N-terminal transmembrane domain, a variable linker, and two highly conserved C-terminal C2 domains (C2A and C2B), possessing variable calcium-binding affinities. A particular isoform Syt III localizes to plasma membrane with six calcium-binding sites. Our previous studies found that Syt III is significantly up-regulated in P4-P6 rat retinal ganglion cells, suggesting that Syt III may play a unique role in regulating synaptic activity in the developing nervous system. However, the molecular mechanism underlying Syt III's regulation of vesicle release remains elusive. In this study, we overexpressed Syt III and Syt III-C2AB* (a mutant harboring the abolished calcium binding

sites) in PC12 cells. At 3 days post transfection, we harvested the cell lysates and performed endogenous co-immunoprecipitation. We found that abolishing the calcium binding sites in Syt III mainly regulated its interaction with SNAP-25 (a t-SNARE), consistent with the localization of Syt III to plasma membrane. Moreover, by comparing with control and Syt III-C2AB*, overexpressing Syt III shortened the duration of fusion pores leading to full dilation, suggesting that calcium binding to Syt III may destabilize fusion pores. These results suggest that Syt III regulates the kinetics of fusion pores and the interaction with SNAP-25 through calcium binding to its C2AB domains.

Disclosures: **Y. Tsai:** None. **Y. Huang:** None. **C. Wang:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Center for Single-Molecule Systems Biology

Title: Inositol pyrophosphates inhibit synaptotagmin-dependent synaptic vesicle exocytosis

Authors: T.-S. LEE[1], J.-Y. LEE[2], J. KYUNG[3], S. PARK[2], S. LEE[2], I. PAVLOVIC[5], B. KONG[6], Y. JHO[7], H. J. JESSEN[8], D.-H. KWEON[6], Y.-K. SHIN[9], S. KIM[4], T.-Y. YOON[1], *S. KIM[2];
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Abstract: Inositol pyrophosphates such as 5-IP_7 (5-diphosphoinositol pentakisphosphate) are highly energetic inositol metabolites containing phosphoanhydride bonds. While inositol pyrophosphates are known to regulate various biological events, including growth, survival and metabolism, the molecular sites of 5-IP_7 action in vesicle trafficking have remained largely

elusive. We here report that elevated 5-IP_7 levels, caused by overexpression of IP_6 kinase (IP6K1), suppressed depolarization-induced neurotransmitter release from PC12 cells. Conversely, IP6K1 depletion decreased intracellular 5-IP_7 concentrations, leading to increased neurotransmitter release. Consistently, knockdown of IP6K1 in cultured hippocampal neurons augmented action potential-driven synaptic vesicle exocytosis at synapses. Employing a fluorescence resonance energy transfer (FRET)-based in vitro vesicle fusion assay, we found that 5-IP_7, but not 1-IP_7, exhibited significantly higher inhibitory activity toward synaptic vesicle exocytosis than did inositol hexakisphosphate (IP_6). Synaptotagmin 1 (Syt1), a Ca^{2+} sensor essential for synaptic membrane fusion, was identified as a molecular target of 5-IP_7. Notably, 5-IP_7 showed a 45-fold higher binding affinity for Syt1 compared with IP_6. In addition, 5-IP_7-dependent inhibition of synaptic vesicle fusion was abolished by increasing Ca^{2+} levels. Thus, 5-IP_7 appears to act through Syt1 binding to interfere with the fusogenic activity of Ca^{2+}. These findings reveal a role of 5-IP_7 as a potent inhibitor of Syt1 in controlling the synaptic exocytotic pathway and expand our understanding of the signaling mechanisms of inositol pyrophosphates.

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Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Topic: B.06. Neurotransmitter Release

Support: NIH grant NS44057

Title: The transmembrane domain of synaptobrevin influences synaptic fusion pore flux in mouse hippocampal neurons

Authors: *C.-W. CHIANG, C.-W. CHANG, M. B. JACKSON;
Dept. of Neurosci., Univ. of Wisconsin Madison, Madison, WI

Abstract: The fusion pore is the first stable intermediate of exocytosis that mediates release, and is believed to be a bifurcation point between full-fusion and kiss-and-run. Studies in endocrine cells have shown that transmembrane domain (TMD) mutations in the SNARE proteins syntaxin and synaptobrevin (Syb) influence flux through the fusion pore. These results suggest that a proteinaceous fusion pore composed of both SNARE protein TMDs connects the vesicle and plasma membrane. To test this model in synaptic transmission we cultured hippocampal neurons

from Syb/cellubrevin double knockout mice and expressed Syb mutants with single tryptophan substitutions in its TMD (residues 97 through 108). Miniature excitatory synaptic currents (mEPSC) were recorded using whole cell patch clamp. Transmitter flux was inferred from the kinetics evaluated with the aid of careful fits to individual events. We identified mutations at position 99, 101, 103, 104, and 105 that reduced the maximum slope during the rising phase of mEPSCs, which represents the highest velocity of efflux through the initial fusion pore. Residues 101 and 103 also have increased rise time. Except for residue 104, these residues had been shown previously to influence flux through endocrine fusion pores. Our results suggest that the Syb TMD forms part of the fusion pore during synaptic vesicle exocytosis, and substitutions in the pore lining residues for amino acid with higher volume hinder the passage of neurotransmitter and slow down release. The comparison with previous studies indicates that endocrine and synaptic release employ very similar molecular machinery in forming fusion pores.

Disclosures: **C. Chiang:** None. **C. Chang:** None. **M.B. Jackson:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Topic: B.06. Neurotransmitter Release

Title: A chimeric approach to studying the Syntaxin Habc domain uncovers roles in exocytosis and Syntaxin trafficking

Authors: *L. A. PARRA, M. T. PALFREYMAN, E. M. JORGENSEN;
Univ. of Utah, Salt Lake City, UT

Abstract: Synaptic vesicle fusion is mediated by the concerted action of Munc18 and SNARE proteins. Munc18 has multiple binding modes with syntaxin. Key to understanding this interaction is the highly conserved three-helix bundle (known as the Habc domain) that is present on the SNARE protein syntaxin. This domain is the major interaction interface between Munc18 and the SNARE proteins. To test the role of the Habc domain and Munc18 proteins *in vivo*, we are analyzing different Habc domain variants using the nematode *C. elegans*. Specifically, we are exchanging the Habc domain of the worm with the respective Habc domains of other species. Using this chimeric approach we hope to identify key components that will help to mechanistically characterize the interaction between the SNAREs and the Munc18 proteins. Previous evidence suggests the interaction of Munc18 and the SNAREs serves a role in trafficking and in exocytosis. Our evidence shows that replacing the worm Habc with the

choanoflagellate *Monosiga brevicollis* (MoBr) Habc does not disrupt trafficking whereas the yeast *Saccharomyces cerevisiae* (SaCe) Habc domain does result in partial trafficking defects. Despite the correct localization, the choanoflagellate Habc chimera animals are still uncoordinated and have dramatic decrease in synaptic vesicle fusion -thus, demonstrating the important role for the Habc domain downstream of trafficking. Interestingly, the roles downstream of trafficking might result from specific interactions between the Habc domain and Munc18. Specifically, the inviability of yeast Habc chimeras can be rescued by expression of Sec1p, the yeast homolog of Munc18. In this combination the viability is restored independent of an observable restoration of wild-type syntaxin localization. I am currently performing further chimeric analyses to understand the physiological implication of syntaxin Habc domain interaction with Munc18. These experiments will parse the roles of Munc18 in the trafficking of syntaxin and the exocytosis of synaptic vesicles.

Disclosures: **L.A. Parra:** None. **M.T. Palfreyman:** None. **E.M. Jorgensen:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Topic: B.06. Neurotransmitter Release

Title: The role of Auxilin in neurosecretion in NGF-treated PC12 Cells.

Authors: *W. LIM;
Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Endocytosis are important transportation of molecules outside to inside of a cell. One of the endocytosis is Clathrin-mediated endocytosis (CME). In neuron CME is an important mechanism for the formation of new vesicles at the presynaptic-terminal and synaptic vesicles recycling. CME starts from assembly proteins then bind to the synaptic vesicle proteins (SNAREs) and phosphoinositides; later recruiting clathrin a protein which will surround and coat the vesicle. After the vesicle fission into the cell de-coating of clathrin layer is required for the content inside of the vesicles to release or uptake into early endosome. Early studies indicated that Hsc70 and Auxilin facilitate in the process of de-coating clathrin layer. Auxilin was shown to be a J cochaperone which—like DnaJ, contains a J domain which binds on Hsc70. Auxilin also contains a clathrin-binding domain it bind to clathrin coat and recruiting Hsc70 this provides the mechanism to drive clathrin-caot disassembly. In this study we focus on expressing auxilin and its mutation forms into NGF-treated PC12 cell to study their effect on endocytosis. To monitor the effect of endocytosis, we use perforated-patch to record the cell capacitance changes,

when cell undergoes more exocytosis the surface areas of the cell membrane will increase and the capacitance will also increase, in contrast when endocytosis is more than exocytosis, the cell membrane surface area will decrease and so the capacitance. By this method we can monitor the living cell capacitance changes and verify the role of different Auxilin domain in endocytosis.

Disclosures: W. Lim: None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Brain & Behavior Research Foundation

International Mental Health Research Organization

Title: Spontaneous neurotransmission driven by non-canonical vesicular SNAREs modulates synaptic plasticity

Authors: *D. C. CRAWFORD, D. M. O. RAMIREZ, B. TRAUTERMAN, L. M. MONTEGGIA, E. T. KAVALALI;
UT Southwestern Med. Ctr., Dallas, TX

Abstract: Most presynaptic vesicles fuse with the plasma membrane after the arrival of an action potential, but some vesicles fuse spontaneously in the absence of such stimulation. Recent studies suggest that stimulus-evoked and spontaneous neurotransmitter release processes are molecularly distinct, so it is possible that these forms of neurotransmission differentially signal to the postsynaptic neuron. To test this hypothesis, we targeted the non-canonical synaptic vesicle SNARE proteins Vps10p-tail-interactor-1a (vti1a) and vesicle-associated membrane protein 7 (VAMP7) to specifically impair spontaneous release events and probe whether loss of these proteins alters synaptic plasticity. In cultured neurons, loss of vti1a and VAMP7 impaired spontaneous high-frequency glutamate release and augmented unitary event amplitudes by reducing postsynaptic eukaryotic elongation factor 2 kinase (eEF2K) activity. In hippocampal

brain slices, loss of vti1a and VAMP7 reduced spontaneous neurotransmitter release, as measured via uptake of antibody against the luminal domain of synaptotagmin 1. Presynaptic, but not postsynaptic, loss of vti1a and VAMP7 in hippocampal slices occluded N-methyl-D-aspartate receptor (NMDAR) antagonist-induced synaptic potentiation in an intact circuit, confirming the role of these vesicular SNAREs in setting synaptic strength. Long-term potentiation, which depends on stimulus-evoked neurotransmission for induction, was not impaired by presynaptic loss of vti1a and VAMP7. Collectively, these results demonstrate that spontaneous neurotransmission signals independently of stimulus-evoked release and is a key regulator of postsynaptic efficacy.

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Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

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Topic: B.06. Neurotransmitter Release

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Howard Hughes Medical Institute

Title: An improved event-driven model of presynaptic dynamics for large-scale simulations

Authors: *J. W. GARCIA[1,2], T. M. BARTOL[1,2], D. J. SPENCER[1,2], T. J. SEJNOWSKI[1,2]; [1]Salk Inst., La Jolla, CA; [2]Howard Hughes Med. Inst., La Jolla, CA

Abstract: Chemical synapses play a central role in neural computation in the brain: the dynamics of probabilistic vesicle release, facilitation, and depression at these synapses strongly determine how information is transferred between neurons. However, the precise computational role of these presynaptic dynamics remains under investigation. To elucidate their role in biological networks, computational simulations with many neurons and synapses require synaptic models with strong realism, efficiency, and versatility. Our model aims to replicate the phenomenology of both biological synapses and validated molecular models of presynaptic function through an efficient event-driven framework that tracks action potential, vesicle release, priming, reuptake, and recycling event times. The model includes multiple mechanisms of asynchronous vesicle release, facilitation, and depression and easily allows features to swap in and out for controlled modelling experiments. It overcomes numerous limitations of previous

models, achieving high realism with minimal sacrifice in computational efficiency, which makes it ideal for studying the role of presynaptic dynamics in large network simulations.

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Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 503.17/J12

Topic: B.06. Neurotransmitter Release

Support: Howard Hughes Medical Institute

Title: Comparing models of synaptic release by combined experimental recordings and neuronal simulation

Authors: *D. SPENCER, J. W. GARCIA, T. M. BARTOL, T. J. SEJNOWSKI;
Salk Inst. CNL-S, La Jolla, CA

Abstract: Computational neuroscience today is moving toward larger and more heterogeneous networks of neurons. These simulations are modeling neurons and their interconnections in greater detail to match improvements in experimental neurophysiology. Inclusion of detailed modeling of synapses in network simulations, however, is lagging due to their large numbers, complex behavior and diversity. Relatively few network simulations today incorporate individual synapses and those that do use simplified facilitation/depression models that release synchronously with the input axonal spikes. Newer synaptic models are emerging that encompass multiple presynaptic release mechanisms and produce asynchronous releases over time spans greater than one second from a single input spike. A problem in these new synaptic models is how to tune their many parameters and how to compare and rate the models for their fidelity. Traditionally, the comparisons have been with in vitro experiments encompassing presynaptic spikes stimulating axons that synapse onto a postsynaptic neuron from which facilitation, depression, and release probability can be calculated and compared. Here we use a simulated postsynaptic neuron and input axons that model in vivo experiments of a controlled behavioral stimulation with simultaneous recording of pre synaptic and postsynaptic neurons. A typical experiment would be simultaneous recordings of mammalian LGN neurons and V1 cortical neurons during stimulation of the eyes by either natural or artificial scenes. We evaluate different synaptic models by driving them with the same experimental presynaptic spike trains and statistically measuring the difference of the simulated postsynaptic output with the in vivo

neuron recordings. This paradigm can also be use to “tune” a synapse model. Using different sets of experimental data, we compare synapse models of varying computational complexity. They are compared for their closeness to the in vivo cortical neuron output as well as for their facilitation, depression, and release profiles.

Disclosures: D. Spencer: None. **J.W. Garcia:** None. **T.M. Bartol:** None. **T.J. Sejnowski:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 503.18/J13

Topic: B.06. Neurotransmitter Release

Support: MCCIG PCIG12-GA-2012-334318

Title: Dmnoa1 function at the *Drosophila* neuromuscular junction

Authors: *R. HABETS;
Leiden Univ. Med. Ctr., Leiden, Netherlands

Abstract: Neurotransmitter release is a process that is critically dependent on mitochondria due to the high energy demand of the vesicle recycling machinery. We isolated mutants in the *Drosophila* homologue of NOA1, which we called DmNOA1, in a screen for mutants with synaptic defects. NOA1 is a circularly permutated GTPase that is conserved from yeast to man and we find DmNOA1 localized at the mitochondria of muscles and neuromuscular junctions. Rescue experiments indicate that DmNOA1 needs to be expressed in all tissue to rescue lethality in the mutants. Interestingly, mutant animals are developmentally delayed, meaning that the larval stage is prolonged to up to 20 days. We find that mitochondrial morphology is normal in mutant animals, but to our surprise found that levels of complex I of the electron transport chain are elevated in *DmNOA1* mutants. Basic synaptic transmission at the larval neuromuscular junction was not altered, yet upon high frequency stimulation excitatory junctional potentials depressed to a greater extend in *DmNOA1* mutant animals. We conclude that DmNOA1 is necessary for correct assembly of complex I and that failure to do so results in synaptic defects in *DmNOA1* mutants.

Disclosures: R. Habets: None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 503.19/J14

Topic: B.06. Neurotransmitter Release

Support: Heart and Stroke (JSD)

CIHR (MBS)

NSERC (MBS)

Title: Distinct functions of cGMP-dependent protein kinase in synaptic function

Authors: *J. S. DASON, A. M. ALLEN, M. B. SOKOLOWSKI;
Univ. of Toronto, Toronto, ON, Canada

Abstract: The *foraging* gene in *Drosophila* encodes a cGMP-dependent protein kinase (PKG). PKG is thought to regulate several aspects of synaptic function, including synaptic plasticity, synaptic vesicle exocytosis and endocytosis, and neurite outgrowth. However, the mechanisms by which PKG regulates these processes are not fully understood. In addition, much of the evidence for its putative role in these processes is based on the use of pharmacological inhibitors. Pharmacological approaches can be limiting because of their non-specific effects and the inability to distinguish between presynaptic, postsynaptic and glial effects. To overcome these limitations, we used a genetic approach to understand the role of PKG in synaptic function. Here, we used a newly created *foraging* null mutant to characterize the synaptic effects of PKG at the *Drosophila* larval neuromuscular junction. We found that the *foraging* null mutant displayed increased nerve terminal growth, increased neurotransmitter release in response to low frequency stimulation and impaired synaptic vesicle endocytosis. Next, we used RNAi to knockdown *foraging* selectively in neurons, glia or muscles to determine where PKG was required for these synaptic effects. We found that glial PKG regulated synaptic growth, presynaptic or postsynaptic PKG regulated neurotransmitter release and presynaptic PKG regulated synaptic vesicle endocytosis. Finally, we used FIAsH-FALI to acutely inactivate PKG and separate its roles in synaptic vesicle exocytosis and endocytosis. Overall, we found that presynaptic, postsynaptic and glial PKG have distinct roles in regulating synaptic growth and synaptic vesicle cycling.

Disclosures: **J.S. Dason:** None. **A.M. Allen:** None. **M.B. Sokolowski:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 503.20/J15

Topic: B.06. Neurotransmitter Release

Support: NIH-1P20GM103653-01A1

Title: Role of D2 auto-receptors in neurotransmitter modulation

Authors: *H. S. DHILLON[1], R. FORMISANO[1], J. CAPLAN[2];
[1]Biol. Sci., Delaware State Univ., Dover, DE; [2]Biol. Sci., Univ. of Delaware, Newark, DE

Abstract: The neurotransmitter dopamine influences a variety of neural processes ranging from reward, cognition and locomotion, both in vertebrate and invertebrate. Several neuropsychiatric disorders including Parkinson's disease, schizophrenia, depression and bipolar disorders are associated with dopamine imbalances. Dopamine signaling is mediated via two sub-types of G-protein coupled receptors (GPCR) receptors, D1-like and D2-like receptors. Dopamine autoreceptors are a sub-type of D2 receptors that are found at both somatodendritic and axonal sites and are suggested to mediate the amount and the timing of dopamine release. In contrast to the complexity of the mammalian CNS, the 302-neuron nervous system of *Caenorhabditis elegans* provides a powerful as well as simpler biological system that has been successfully used to link genes, individual neurons, and neural circuits to specific behaviors. The phylogenetic conservation of *C. elegans* genes involved in DA signaling provides a pragmatic model to unravel DA regulatory mechanisms. There are 8, clearly identifiable DA neurons in the *C. elegans* hermaphrodite and 4 confirmed *C. elegans* DA receptors. We are characterizing its D2-like receptor DOP-2 to understand the role of autoreceptors in modulation of dopamine signaling in movement control and non-associative learning. Previously, we have shown that DOP-2, which is expressed in all eight *C. elegans* dopaminergic neurons, physically interacts with GPA-14, an inhibitory G-alpha subunit (Pandey et al., 2012, *J Mol Signal 7: 3*). We have also shown that both *dop-2* and *gpa-14* deletion mutants habituate at a significantly faster rate as compared to wild-type worms (Mersha et al., 2013, *Behav Brain Funct. 9:16*). Our current work is focused towards analyzing synaptic vesicles release in *dop-2* mutants using fluorescence recovery after photobleaching (FRAP) to monitor neurotransmitter vesicle release activity at specific synapses in live animals. Our preliminary results indicate that loss of *dop-2* directly influences the rate of synaptic vesicle fusion potentially suggesting that the basal excitation of dopaminergic neurons may be elevated in *dop-2* animals. In parallel we are also studying the effect of *dop-2* deletion on movement control using swimming induced paralysis assay (Hardaway et al., 2012, *G3 Bethesda 2:961*), in efforts towards understanding the functional contribution of D2 auto-receptors on

motor activity. Results from our experiments have the potential to provide basic science foundations towards understanding disorders of movement control.

Disclosures: **H.S. Dhillon:** None. **R. Formisano:** None. **J. Caplan:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 503.21/J16

Topic: B.06. Neurotransmitter Release

Support: Wellcome Trust-DBT India Alliance

INSPIRE Fellowship

Title: Activity dependent vesicle recycling at the CA3-CA1 synapse contributes to synaptic plasticity

Authors: ***V. MUDIGONDA**, S. NADKARNI;
Dept. of Biol., Indian Inst. of Sci. Educ. and Res., Pune, India

Abstract: In the hippocampal CA3-CA1 synapse, a well studied synapse for plasticity mechanisms, the pool of vesicles available for immediate release (readily releasable pool, or RRP) is typically limited to a small number (7-10). Recruitment of vesicles to RRP takes place via two distinct reservoirs. Each of these have a characteristic time scale of refilling and an associated number of vesicles. In a high frequency regime of activity, usually required to induce plasticity in these synapses, the RRP may quickly run out of its vesicle resource. Given that the vesicle recycling timescales (order of seconds) are much slower compared to the ongoing electrical activity, how do small synapses cope up with high activity demands? Recent experimental studies suggest that the recycling rates are indeed modulated in order to keep up with vesicle release needs. One of the ways vesicles achieve faster recycling rates is exocytosis sans loss of vesicle membrane identity (kiss and run mechanism). This enabling mechanism has specifically been observed to play a role for high frequency stimulus at CA3-CA1 synapse. The identity of several molecular players which coordinate these processes at the presynaptic terminal have been elucidated, however a quantitative framework to model the dynamics and its effect on plasticity thus far does not exist. Here, we develop a detailed kinetic model with 3 distinct pools for synaptic vesicles at the CA3-CA1 synapse. Our model takes into account kiss and run and full fusion forms of vesicle recycling. We include a frequency and calcium dependence for the recycling rates in accordance with reported results. We elucidate synaptic

vesicle dynamics under various plasticity protocols. Furthermore, we explore how changes in vesicle pool sizes can alter synaptic plasticity. The computational framework developed here could be generalized to other synapses like the calyx of Held and allow us to have better quantitative understanding of mechanisms implicated in presynaptic plasticity.

Disclosures: V. Mudigonda: None. **S. Nadkarni:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

Location: Halls B-H

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Program#/Poster#: 503.22/J17

Topic: B.06. Neurotransmitter Release

Support: NIH Grant NS082573

MDA grant MDA236717

Title: Fatigue in rapsyn-deficient zebrafish reflects defective transmitter release

Authors: *H. WEN[1], M. MCGINLEY[2], J. M. HUBBARD[3], W.-C. WANG[1], P. BREHM[1]; [1]The Vollum Inst., Oregon Hlth. and Sci. Univ., Portland, OR; [2]Texas Children's Hosp., Houston, TX; [3]Inst. du Cerveau et de la Moelle épinière, Paris, France

Abstract: Generally associated with a drop in release probability among release sites, synaptic depression at the zebrafish neuromuscular junction results instead from the dropout of a subset of slow release sites that require seconds to recover. This determination was based on multinomial variance analysis of evoked endplate current (EPC) amplitudes obtained with paired motor neuron-muscle recordings. Our analysis indicated a release probability near unity at frequencies <0.2 Hz and an average of 14 functional release sites. These consisted of two kinetically distinct groups that are >60 fold different in their recovery rates, with each accounting for approximately half of the total release site number. At frequencies above 1 Hz the number of functional release sites dropped to half due to the inability of the slow sites to reload. The midpoint of steady state depression was resistant to change between 20 and 100 Hz due to the fast time constant of recovery of the remaining sites. The steady state level of transmission was maintained solely by the fast release sites, which recovered within <45 ms. We applied the analysis to a mutant line of zebrafish which serves as a model for human rapsyn deficient myasthenic syndrome. As with most myasthenic syndromes in human, the fish underwent rapid use-dependent muscle fatigue, the basis for which has remained unresolved. We now show that the fatigue results from exaggerated synaptic depression compared to wild type fish. As with wild type there are two

groups of kinetically distinct release sites, but in the mutant line the recovery rate specifically for the fast sites is greatly compromised. Thus, the postsynaptic mutation in rapsyn leads to lowered receptor density but also impacts the presynaptic release. The collective actions of defective presynaptic release and postsynaptic responsiveness lead to fatigue onset that is frequency dependent.

Disclosures: H. Wen: None. **M. McGinley:** None. **J.M. Hubbard:** None. **W. Wang:** None. **P. Brehm:** None.

Poster

503. Exocytosis and Endocytosis: Mechanisms and Regulation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 503.23/J18

Topic: B.06. Neurotransmitter Release

Support: NSF LSAMP Bridge to Doctorate Fellowship

Title: Behavioral consequences of point mutations in the vesicular acetylcholine transporter

Authors: *D. WHITE, JR, A. WALLACE, O. AKRINSOLA, S. BOPPANA, H. LAWAL; Biol. Sci., Delaware State Univ., Dover, DE

Abstract: The neurotransmitter acetylcholine (ACh) is involved in critical organismal functions as locomotion, learning and memory. Therefore, decline in this neurotransmitter system is a key underlying factor in movement and cognitive deficits. The vesicular acetylcholine transporter (VAChT) is responsible for packaging ACh into synaptic vesicles for exocytotic release. Mutations in this protein ultimately diminish locomotion whereas complete loss of function of VAChT is fatal. The direct role of altered acetylcholine release and its association with impairment or enhancement of cognitive functions is still not fully understood**.** We hypothesize that point mutations in VAChT will cause age-related deficits in cholinergic-mediated behaviors such as locomotion and learning and memory. Using *Drosophila melanogaster* as a model system, we have generated several mutations within VAChT and observed its effect on behaviors such as lifespan and locomotion. Here we report that VAChT point mutants show age-dependent defects in locomotion ability; and certain mutant alleles display a shorter lifespan. Moreover, we present preliminary studies on the effect of these mutants on courtship conditioning, a well-described assay for learning and memory in *Drosophila*. We also show the expression pattern of VAChT in both wildtype and VAChT point mutants. Together, these results demonstrate that central cholinergic release is important for the regulation of behavioral performance in *Drosophila*. In future studies, we will test methodologies to effectively rescue these deficits with

implications for intervention strategies to treat cholinergic deprived disorders such as Alzheimer's disease.

Disclosures: **D. White:** None. **A. Wallace:** None. **O. Akrinsola:** None. **S. Boppana:** None. **H. Lawal:** None.

Poster

504. Short-Term Plasticity

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 504.01/K1

Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS032405

Nancy Lurie Marks Research Fellowship

Title: How does synaptotagmin 7 contribute to synaptic facilitation?

Authors: ***S. L. JACKMAN**, J. TURECEK, W. G. REGEHR;
Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Many neuronal synapses exhibit facilitation, a form of short-term enhancement in which the amount of neurotransmitter release increases with each subsequent presynaptic action potential. While synchronous neurotransmitter release is triggered during action potentials when local, high-concentrations of Ca^{2+} bind to the fast synaptotatgmin (Syt) isoforms Syt1 and Syt2, facilitation is driven by the smaller, longer-lasting Ca^{2+} signals between action potentials known as residual Ca^{2+}. Thus it was proposed that a specialized Ca^{2+} sensor, distinct from the fast synaptotagmin isoforms, binds residual Ca^{2+} to produce facilitation. We recently showed that Syt7 is required for facilitation at several central synapses. In Syt7-knockout mice, facilitation is eliminated even though the initial probability of release and the presynaptic residual calcium signals are unaltered. Yet how Syt7 produces facilitation remains a mystery. To explore the mechanism by which Syt7 contributes to facilitation, we constructed a biophysical model that incorporates the measured Ca^{2+}-affinities and lipid binding properties of Syt1 and Syt7. Our model recapitulates the behavior of several well-characterized synapses, and may also provide insight into the mechanism by which synaptotagmins trigger neurotransmitter release.

Disclosures: **S.L. Jackman:** None. **J. Turecek:** None. **W.G. Regehr:** None.

Poster

504. Short-Term Plasticity

Location: Halls B-H

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Program#/Poster#: 504.02/K2

Topic: B.08. Synaptic Plasticity

Support: DFG / SFB889

University of Goettingen Medical Center

DFG / CNMPB

Title: The presynaptic protein Mover regulates frequency facilitation at hippocampal mossy fibre terminals

Authors: J. S. VIOTTI, H. POFANTIS, A. K. AKULA, M. ARNDT, *T. DRESBACH;
Univ. of Goettingen Med. Sch., Goettingen, Germany

Abstract: Few proteins in the neurotransmitter release machinery escape the evolutionary conservation that makes synapses among nematodes, flies, mice and man quite similar. Mover is one of these rare exceptions. Mover is a vertebrate-specific, synaptic vesicle-bound phosphoprotein. It binds both the highly conserved Calmodulin and the vertebrate-specific active zone scaffolding protein Bassoon. Moreover, Mover is highly expressed at some and below detection limit at other synapses, indicating that it may regulate synaptic function at subsets of synapses. In addition, Mover is strongly upregulated in the anterior cingulate cortex in post-mortem brains of schizophrenic patients, raising the possibility that it is upregulated in response to - or as a cause of - aberrant neuronal activity. Knock-down of Mover at the calyx of Held increases the rate of vesicle reloading after synaptic depression, as well as the calcium sensitivity and probability of release, revealing a role for Mover in regulating transmitter release at this synapse.
In this study, we have used a Mover knockout mouse line in combination with imaging and electrophysiology to understand the role of this protein in synaptic transmission. Knockout of Mover increases frequency facilitation, paired-pulse ratio and high-frequency facilitation in the hippocampal mossy fiber - to CA3 synapse, but not in the Schaffer collateral - to CA1 synapse. These data suggest that Mover boosts release probability and dampens frequency facilitation in hippocampal mossy fibre terminals.
In cultured neurons, Mover is upregulated when global activity is increased and downregulated when activity is decreased.
These discoveries, together with its role in the Calyx of Held, suggest that Mover is an activity-dependent, synapse-specific regulator of presynaptic plasticity. Moreover, they suggest that a) Mover has distinct roles at different synapses; b) generally acts to dampen the extent of

presynaptic events; c) acts as a brake that can be released during low activity. At the hippocampal mossy fibre terminal, activity-dependent regulation of Mover could increase the dynamic range for the induction of frequency facilitation and working memory.

Disclosures: **J.S. Viotti:** None. **H. Pofantis:** None. **A.K. Akula:** None. **M. Arndt:** None. **T. Dresbach:** None.

Poster

504. Short-Term Plasticity

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 504.03/K3

Topic: B.08. Synaptic Plasticity

Support: NIH R01MH095248

NIH T32MH067564

Title: Sex difference in the requirement of different Ca^{2+} sources to initiate estradiol-induced excitatory synaptic potentiation in the hippocampus

Authors: ***A. JAIN**[1], C. S. WOOLLEY[2];
[2]Neurobio., [1]Northwestern Univ., Evanston, IL

Abstract: 17β-estradiol (E2) acutely modulates neuronal physiology in the hippocampus. One acute effect of E2 is to potentiate excitatory synaptic transmission at CA3-CA1 synapses. Whole-cell recordings in hippocampal slices from rats show that bath application of E2 (100nM) potentiates excitatory postsynaptic currents (EPSCs) in ~60% of CA1 pyramidal cells in both females and males. However, this occurs through distinct underlying mechanisms in each sex. Preliminary results show that protein kinase A (PKA) is required to initiate E2-induced synaptic potentiation in females but not in males. Because increased intracellular Ca^{2+} can activate kinases to initiate synaptic potentiation, here we investigated whether either of two principal sources of increased intracellular Ca^{2+}, L-type Ca^{2+} channels or Ca^{2+} release from internal stores, are required for E2-induced synaptic potentiation, and whether this differs by sex. We used nifedipine (10μM) to block L-type Ca^{2+} channels and thapsigargin (1μM) to deplete internal Ca^{2+} stores.
Nifedipine by itself had no effect on EPSCs in either sex. Thapsigargin produced a transient increase in EPSC amplitude in both sexes and E2 was applied after EPSC amplitude returned to baseline. We found that, in females, E2-induced synaptic potentiation was inhibited completely by either nifedipine (13 of 13 cells) or thapsigargin (9 of 9 cells). In contrast, in males, neither

nifedipine nor thapsigargin alone was sufficient to inhibit this potentiation. In males, E2 potentiated EPSCs in nifedipine by 68±7% in 9 of 15 cells and in thapsigargin by 56±6% in 6 of 10 cells, both statistically similar compared to potentiation by E2 alone (81±4%, 11 of 18 cells) (unpaired t-test, nifedipine: p=0.26, thapsigargin: p=0.16). However, when L-type Ca^{2+} channels were blocked and internal Ca^{2+} stores were depleted together in males, E2-induced synaptic potentiation was completely inhibited (9 of 9 cells). Thus, while both L-type Ca^{2+} channels and Ca^{2+} release from internal stores are involved in initiation of E2-induced potentiation in both sexes, one Ca^{2+} source can compensate the other in males, whereas in females, both are required to initiate synaptic potentiation. Further experiments will investigate whether the requirement for both L-type Ca^{2+} channels and Ca^{2+} release from internal stores in females is related to the female-specific requirement of PKA in E2-induced synaptic potentiation. Understanding differences between males and females in E2-induced synaptic modulation may provide insight into mechanisms that underlie sex differences in the prevalence and/or symptoms of neuropsychiatric disorders that vary by sex.

Disclosures: A. Jain: None. **C.S. Woolley:** None.

Poster

504. Short-Term Plasticity

Location: Halls B-H

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Program#/Poster#: 504.04/K4

Topic: B.08. Synaptic Plasticity

Support: ABI Grant 000482-00001

Title: Inter-hemispheric modulation of TMS-evoked potentials following intermittent theta burst stimulation to motor cortex

Authors: *M. GANNON, S. LONG, N. PARKS;
Univ. of Arkansas, Fayetteville, AR

Abstract: Transcranial magnetic stimulation (TMS) produces a transient magnetic field that is capable of activating cortical tissue through induction. Repetitive TMS (rTMS) uses repeated administration of TMS pulses and has been reliably shown to produce changes in the state of cortical excitability outlasting the time of stimulation. One such protocol that has demonstrated states of increased excitability is intermittent theta burst stimulation (iTBS). This method applies high-frequency bursts (50Hz) of pulses every 200 ms in trains of ten bursts. Protocols such as these have found use in clinical domains for the treatment of a variety of neurological and psychological disorders. However, there are still many unknowns regarding the

neurophysiological changes that accompany this plasticity. Here, we sought to investigate neurophysiological mechanisms of iTBS-induced plasticity in motor cortex using EEG simultaneously recorded with TMS pulses. Further, we sought to examine inter-hemispheric changes in motor cortex excitability attributable to iTBS. That is, we examined if iTBS conducted over right motor cortex would lead to measureable changes in excitability indices of left motor cortex. We administered a standard iTBS protocol to subjects' right motor cortex and examined changes in cortical excitability immediately following application and thirty minutes later. We quantified changes in right and left motor cortex excitability with measurements of TMS-evoked potentials (TEPs) and motor-evoked potentials (MEPs) elicited through blocks of single pulse TMS. We compared the effects of the iTBS condition to those found in a control condition where a sham version of iTBS was administered. Results indicate differential modulations of cortical TEPs between hemispheres. The N100 component was enhanced in the iTBS condition across time in the right hemisphere, while there was suppression of the P30 component across time in the left hemisphere.

Disclosures: M. Gannon: None. **S. Long:** None. **N. Parks:** None.

Poster

504. Short-Term Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant 5R01GM105696

NW Mitochondrial Research Guild

Title: Isoflurane inhibits excitatory neurotransmission in the anesthetic hypersensitive mouse mutant, Ndufs4(KO)

Authors: *P. I. ZIMIN, C. B. WOODS, P. G. MORGAN, M. M. SEDENSKY;
Ctr. for Developmental Therapeut., Seattle Children's Res. Inst., Seattle, WA

Abstract: INTRODUCTION: Mitochondrial complex I dysfunction is linked to volatile anesthetic sensitivity in nematodes, mice and children[1-3]. Mice with loss of a mitochondrial complex I subunit, NDUFS4, are very hypersensitive to volatile anesthetics (VAs).[1] Limiting NDUFS4 loss to a subset of glutamatergic neurons recapitulated the total knock-out (KO) VA hypersensitivity phenotype. Exposure to 0.6% isoflurane, which anesthetizes KOs but not controls, selectively depressed spontaneous excitatory neurotransmission in KO CA1 neurons

(submitted for publication). Here we investigated excitatory neurotransmission under conditions of high energetic demand caused by high frequency stimulation (HFS).
METHODS: All studies were approved by our IACUC. Evoked field excitatory postsynaptic potentials (fEPSPs) were recorded from CA1 region of coronal mouse brain slices. Fibers were stimulated every 30s for baseline activity and for at least 60min following HFS, which consisted of 3 trains of 100Hz delivered at 20s intervals. In some experiments isoflurane-containing solution was superfused for 40min prior to HFS, and for the duration of the experiment.
RESULTS: HFS failed to induce potentiation in KO slices within 2min, while control slices showed potentiation to ~150%. By ~10min, KO and control slopes of fEPSPs displayed very similar potentiation, of ~140%, which gradually decreased to ~120% at 60min. 0.6% isoflurane exposure initially decreased slopes of fEPSPs in the KO to only 20% of baseline, gradually increasing to match potentiation in control slices of about 120% over 20min. There were no differences between KO and control in the slopes of fEPSPs during HFS in the absence of isoflurane. In 0.6% isoflurane the KO preparations displayed much lower slopes in the first 50msec in the second and third train of HFS. 0.6% isoflurane corresponds to ~1.5 MAC for the KO. We tested 1.5 MAC isoflurane (1.8%) in controls. Isoflurane reduced fEPSPs to ~25% of baseline at 30s post-HFS, recovering to ~80% by 15min, without further changes.
CONCLUSIONS: Under energetically demanding conditions, isoflurane markedly inhibited the ability of a mitochondrial mutant to recover excitatory synaptic transmission. HFS in our model uncovered selective differences in *Ndufs4(KO)* neuronal function that are consistent with whole animal data for both the global KO and cell specific loss of this protein. Depression of mitochondrial function by isoflurane may limit effective neurotransmission in key circuits responsible for responses to VAs in both normal animals and mitochondrial mutants.
REFERENCES: 1. PLoS One. 2012. 7: e42904 2. Anesth. 2002. 96: 1268 3. Anesth. 1999. 90: 545-554

Disclosures: **P.I. Zimin:** None. **C.B. Woods:** None. **P.G. Morgan:** None. **M.M. Sedensky:** None.

Poster

504. Short-Term Plasticity

Location: Halls B-H

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Program#/Poster#: 504.06/K6

Topic: B.08. Synaptic Plasticity

Support: CIHR

OMHF

Title: Physiological role of bk channels in synaptic plasticity associated with cognitive function

Authors: ***T. ZAMAN**[1], M. SMOKA[2], S. SCHMID[1];
[1]Anat. and Cell Biol., Schulich Sch. of Med. & Dent., London, ON, Canada; [2]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Effective sensory filtering is critical for the healthy brain as it is believed to reduce the processing demands placed on advanced circuitry. Disruption of sensory filtering, as observed in autism and schizophrenia, can be measured as a deficit in habituation of acoustic startle response. In recent years, evidence has accumulated that mechanisms that influence synaptic transmission are involved in habituation. The aim of this study is to investigate the physiological role of calcium and voltage-activated potassium channel (BK channel) in the synaptic plasticity underlying acoustic startle habituation using immunofluorescence, electrophysiological and voltage-sensitive dye imaging tools. We hypothesize that activation and possibly phosphorylation of BK channels in auditory afferents that synapse onto startle mediating caudal pontine reticular nucleus (PnC) giant neurons may play a critical role in the synaptic depression related to startle habituation. Our immunofluorescent staining revealed that BK channels are located on glutamatergic terminals synapsing on PnC neurons. Patch-clamp recordings in rat brainstem slices show that repeated activation of auditory synapses on PnC neurons by short trains of action potentials leads to synaptic depression that is significantly reduced by bath perfusion of both BK channel blocker paxilline and CaMKII phosphorylation inhibitor KN93. Voltage sensitive dye imaging of the brain slice that shows directional flow, magnitude of neuronal activity and consequent plasticity in the PnC region further strengthens the association of BK channels with synaptic depression. In summary, this study provides compelling evidence that BK channels are critical for synaptic plasticity underling sensory filtering that is linked to cognitive function.

Disclosures: **T. Zaman:** None. **M. Smoka:** None. **S. Schmid:** None.

Poster

504. Short-Term Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NINDS grant NS22625

Title: Differential regulation of short-term presynaptic plasticity by calcium channels and calcium sensor proteins in excitatory and inhibitory synapses in the hippocampus

Authors: *E. NANOU, W. A. CATTERALL;
Pharmacol., Univ. of Washington, Seattle, WA

Abstract: Regulation of $Ca_V2.1$ channels by specific calcium sensor (CaS) proteins mediates different patterns of synaptic facilitation vs. rapid synaptic depression in cultured neurons that transiently express $Ca_V2.1$ and CaS proteins. Introducing the mutation IM-AA into the CaS binding site (IM motif) in the C-terminus of $Ca_V2.1$ channels in mice blocks synaptic facilitation in excitatory synapses in single-neuron microcultures. Here we compare the effects of this mutation in three synapses in hippocampal slices: Schaffer Collaterals (SC) to CA1, SC to parvalbumin (PV)-expressing interneurons, and PV interneurons to CA1. In excitatory SC-CA1 synapses, the IMAA mutation reduced synaptic facilitation in response to paired-pulse stimuli by 50%, without an effect on miniature postsynaptic currents, and shifted the pattern of synaptic plasticity in response to trains of stimuli such that both synaptic facilitation and rapid depression were slowed. In excitatory SC-PV synapses, we also found that paired-pulse facilitation was reduced by 50% and both facilitation and rapid depression were slowed in trains of stimuli. Thus, the IM-AA mutation in presynaptic $Ca_V2.1$ channels affected these two different excitatory synapses formed by SC presynaptic nerve terminals in a similar manner. In contrast, the IM-AA mutation affected only rapid synaptic depression in inhibitory PV-CA1 synapses. Synaptic depression dominated at all inter-stimulus tested (20-200 ms). Surprisingly, we found that the IM-AA mutation abolished rapid synaptic depression at inter-stimulus intervals from 50 to 200 ms. We also examined synaptic plasticity in response to trains of action potentials at different frequencies (5-50 Hz). WT synapses exhibited synaptic depression in response to trains at all frequencies tested. Surprisingly again, rapid synaptic depression was abolished in IM-AA synapses at frequencies of 5-20 Hz and significantly slowed at 50 Hz**.** Altogether, our studies show that $Ca_V2.1$/CaS regulation is important for the balance of excitatory and inhibitory circuits, because prevention of CaS regulation slowed development of both facilitation and depression in these excitatory synapses while it only slowed depression in these inhibitory synapses.

Disclosures: E. Nanou: None. **W.A. Catterall:** None.

Poster

504. Short-Term Plasticity

Location: Halls B-H

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Topic: B.08. Synaptic Plasticity

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Boehringer Ingelheim Fonds

Title: Pkc is not the ca sensor for ptp at ca1 synapses

Authors: *C.-C. WANG[1], C. WEYRER[1,2], M. PATURU[1], D. FIORAVANTE[1,3], W. REGEHR[1];
[1]Dept. of Neurobio., Harvard Med. Sch., Boston, MA; [2]Develop. & Neurosci., Univ. of Cambridge, Cambridge, MA; [3]Ctr. for Neurosci., Univ. of California at Davis, Davis, CA

Abstract: Post-tetanic potentiation (PTP) is a widespread form of short-term synaptic plasticity in which a period of elevated presynaptic activation leads to synaptic enhancement that lasts tens of seconds to minutes. A leading hypothesis for the mechanism of PTP is that tetanic stimulation elevates presynaptic calcium that in turn activates calcium-dependent Protein Kinase C (PKC) isoforms to phosphorylate targets and enhance neurotransmitter release. Previous pharmacological studies have implicated this mechanism in PTP at hippocampal synapses, but the results are controversial. Here we combine genetic and pharmacological approaches to determine the role of classic PKC isoforms in PTP. We find that PTP is unchanged in PKC triple knockout (TKO) mice in which all calcium-dependent PKC isoforms have been eliminated (PKCα, PKCβ, and PKCγ). We confirm previous studies and find that in wildtype mice 10 μM of the PKC inhibitor GF109203 eliminates PTP and the PKC activator PDBu enhances neurotransmitter release and occludes PTP. However, we find that the same concentrations of GF109203 and PDBu have similar effects in TKO animals. We also show that 2 μM GF109203 does not abolish PTP even though it inhibits the PDBu-dependent phosphorylation of PKC substrates. We conclude that at the CA3 to CA1 synapse Ca^{2+}-dependent PKC isoforms do not serve as calcium sensors to mediate PTP.
1

Disclosures: C. Wang: None. **C. Weyrer:** None. **M. Paturu:** None. **D. Fioravante:** None. **W. Regehr:** None.

Poster

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People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement n° [291734]

Title: Plasticity-dependent, full detonation at hippocampal mossy fiber-CA3 pyramidal neuron synapses

Authors: N. P. VYLETA[1,2], *C. BORGES-MERJANE[2], X. ZHANG[2], P. JONAS[2];
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Abstract: “Detonator” or “teacher” synapses directly control the activity of postsynaptic targets in the absence of spatial summation. The hippocampal mossy fiber synapse on CA3 pyramidal neurons is thought to be a “conditional detonator” that discharges postsynaptic targets after repetitive activity (Henze et al., 2002, Nat. Neurosci. 5:790-795). The “conditional” status implies that burst activity in granule cells is strictly required for detonation. Whether a single action potential in a single mossy fiber bouton can trigger spikes in CA3 pyramidal neurons remains unknown. Presynaptic facilitation has been identified as a mechanism underlying conditional detonation. However, mossy fiber synapses also exhibit short-term synaptic enhancement with uniquely large and persistent post-tetanic potentiation (PTP). Here we investigated whether PTP could convert mossy fiber synapses from subdetonator into full detonator mode. In order to determine the efficacy of individual connections, we used our recently developed technique to selectively and noninvasively stimulate single mossy fiber presynaptic terminals (Vyleta et al., 2014, Science 343:665-670). Single mossy fiber terminals were stimulated in the tight-seal bouton-attached configuration and postsynaptic CA3 pyramidal neurons were simultaneously recorded in the whole-cell mode at ~33°. Bouton-attached stimulation enabled precise control of electrical activity, as verified by action currents in the presynaptic terminal. We first examined the properties of synaptic transmission evoked by single presynaptic action potentials. Single unitary EPSPs failed to reliably induce action potential initiation in postsynaptic CA3 pyramidal neurons under control conditions, with a mean probability of only 0.12 ± 0.08. We then measured the probability of postsynaptic action potential initiation before and after high-frequency stimulation (HFS, 100 stimuli delivered at 100 Hz), a paradigm that reliably induces PTP. Intriguingly, HFS substantially increased the probability of spiking for the first stimulus, from 0.14 ± 0.09 under control conditions to 0.71 ± 0.18 at the peak and to 0.51 ± 0.16 in a 100-s time window following HFS (22 to 122 s; 7 pairs; P = 0.03). The enhanced detonation decayed with a time constant of 67 s. Thus, PTP converted mossy fiber synapses from subdetonator into full detonator mode for an extended time period. In vivo recordings from awake head-fixed mice revealed that granule cells occasionally fire bursts of >10 action potentials during spatial navigation. Thus, plasticity-induced full detonation may play a role for information processing in the hippocampal network in vivo.

Disclosures: **N.P. Vyleta:** None. **C. Borges-Merjane:** None. **X. Zhang:** None. **P. Jonas:** None.

Poster

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Max Planck Society

Title: State-dependent alteration in $K_v3.4$ channel availability drives flexible synaptic signaling dependent on somatic subthreshold depolarization

Authors: ***M. J. ROWAN**[1], M. LEITGES[2], J. M. CHRISTIE[1];
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Abstract: In many classes of neurons, electrotonic spread of subthreshold depolarizing potentials can reach distant presynaptic specializations in the axon resulting in enhancement of action potential (AP) evoked synaptic transmission. This analog-to-digital facilitation may involve modulation of voltage-gated channels involved in shaping the presynaptic AP, but has also been suggested to result from altered vesicular priming. Thus the molecular mechanisms contributing to analog facilitation of AP-evoked release remain unresolved. We used two-photon (2P) voltage-sensitive dye imaging, patch-clamp recordings from presynaptic boutons, overexpression of tagged voltage-gated channels and genetically modified mice to examine mechanisms contributing to analog signaling in the unmyelinated axons of molecular layer interneurons (MLIs) in the cerebellum. We find that subthreshold somatic depolarization spreads throughout a large extent of the axon arbor resulting in rapid broadening of APs at *en passant* boutons. However subthreshold depolarization did not alter AP duration at the axon initial segment (AIS), indicating a subcellular locus for this form of plasticity within MLI axons. We found that pharmacological blockade of K_v3 channels occluded AP broadening following subthreshold depolarization and, furthermore, that AP broadening was completely abolished in mice lacking rapidly inactivating $K_v3.4$ subunits. Nonuniform expression of axonal AP broadening was due to the segregated expression of $K_v3.4$ channels at presynaptic specializations and their absence in the AIS. Interestingly AP broadening varied considerably between nearby boutons within the same axon, likely due to heterogeneous densities of $K_v3.4$ among nearby

boutons. Following depolarization, wider presynaptic spikes resulted in greater AP-evoked Ca^{2+} influx at boutons and enhanced synaptic transmission. Previous work suggested analog enhancement of release was due to Ca^{2+}-dependent activation of PKC. However, analog enhancement of synaptic transmission was completely abolished in $K_v3.4$ KO mice but unaffected in mice lacking the three classical Ca^{2+}-dependent PKC isoforms (triple KO). Together our results indicate that analog-to-digital facilitation is due to changes in the presynaptic AP waveform in MLIs, and introduce a novel mechanism necessary for analog-to-digital facilitation in a GABAergic interneuron.

Disclosures: M.J. Rowan: None. **M. Leitges:** None. **J.M. Christie:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: BMBF grant 01EO1401 (German Center for Vertigo and Balance Disorders)

Title: Short-term synaptic depression can increase the information rate at a release site

Authors: *M. SALMASI[1,2,3], A. LOEBEL[6,4], S. GLASAUER[5,2,3,1,6], M. STEMMLER[6,4]; [1]Ctr. for Sensorimotor Res., [2]German Ctr. for Vertigo and Balance Disorders, [3]Grad. Sch. of Systemic Neurosciences, [4]Dept. of Biol. II, [5]Dept. of Neurol., Ludwig-Maximilian Univ., Munich, Germany; [6]Bernstein Ctr. for Computat. Neurosci., Munich, Germany

Abstract: Short-term synaptic depression is a ubiquitous feature of neuronal activity. A central functional role of depression is hypothesized to be the modulation of the synaptic information rate [1-3]. However, quantifying the mutual information rate of the overall synaptic transmission process in the presence of short-term dynamics is challenging. We consider therefore a bottom-up approach, and analytically calculate the information rate of a single synaptic release site in the presence of depression. The release site's response dynamics is captured by a general multi-state model, in which each state corresponds to a distinct binary asymmetric channel that depends on the previous *L* time steps of the release site's history (Fig. 1A). With each successful release, the spike-evoked and spontaneous release probabilities depress fractionally more; and in the quiescent intervals, in which no vesicle is released, the probabilities recover gradually back to their default values.
We prove that the mutual information rate of the release site with depression is equal to the statistical average over the information rates of all of its constituent states. We also calculate an

energy-normalized information rate measure, which considers the amount of energy consumed by each vesicle release. We show that depression can increase both the mutual and energy-normalized information rates of the release site, if the spontaneous release depresses more than the spike-evoked release. An additional regime of synaptic parameters exists for which depression increases the energy-normalized information rate, while the mutual information rate decreases (Fig. 1B). However, if spontaneous and spike-evoked release depress equally, depression *invariably* impairs the energy-normalized information rate of the release site. Equal depression would be the default assumption for the synapse. But this assumption implies that the biological system does not fulfill energy-efficient information transfer.

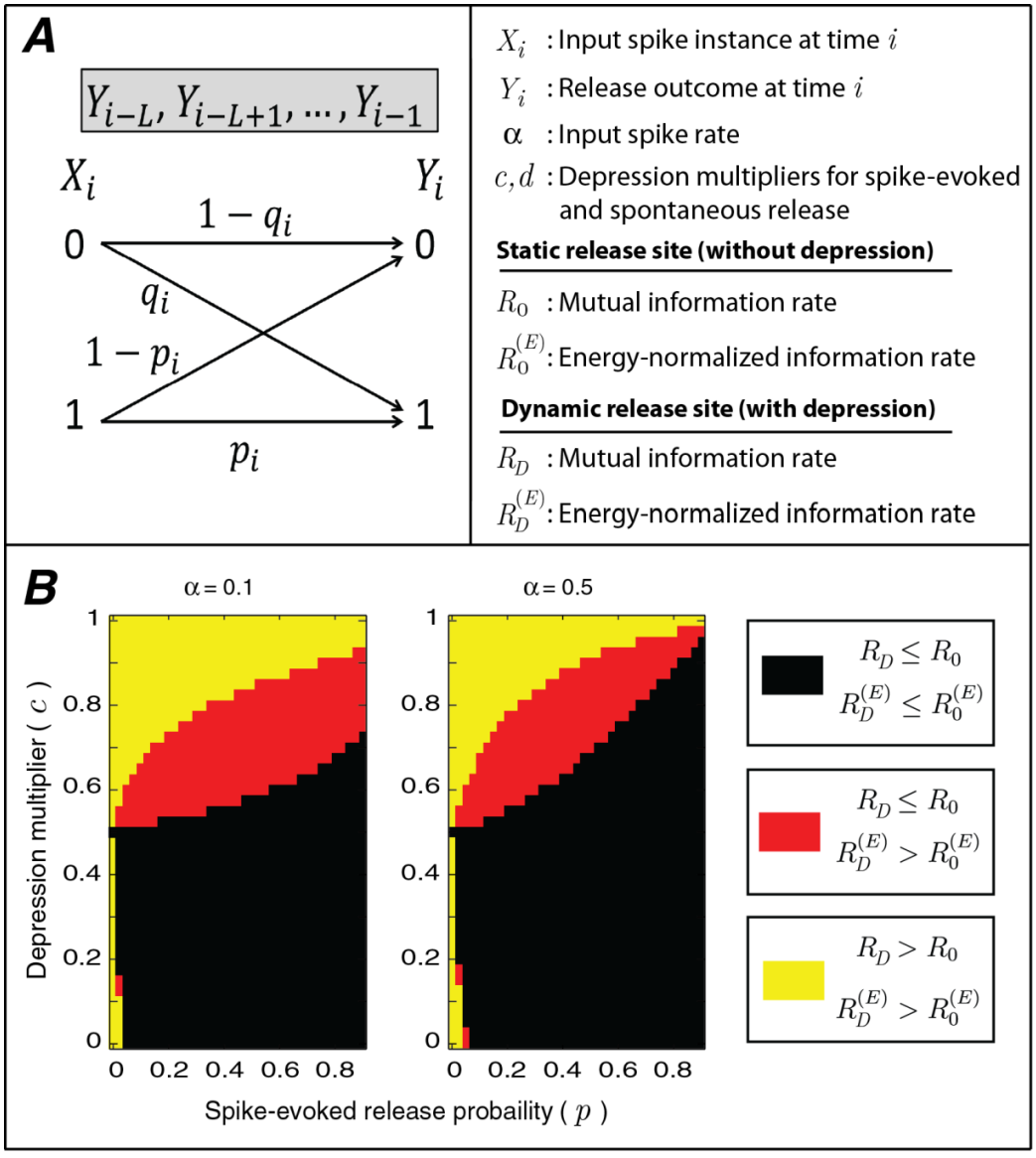

Figure 1: A) The dynamic release site (with depression) modeled by a binary asymmetric channel with memory. B) The impact of depression on the information rate and energy-normalized information rate of the release site. The depression multiplier of spontaneous release is fixed at d=0.5.

1- Fuhrmann et al., J NEUROPHYSIOL, 2002.
2- Goldman, NEURAL COMPUT, 2004.
3- Salmasi et al., BMC NEUROSCI, 2015.

Disclosures: **M. Salmasi:** None. **A. Loebel:** None. **S. Glasauer:** None. **M. Stemmler:** None.

Poster

504. Short-Term Plasticity

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Topic: B.08. Synaptic Plasticity

Support: National Science Foundation Grant IOS1051605 (G. A. Lnenicka)

Title: Activity-dependent postsynaptic potentiation studied at the *Drosophila* larval neuromuscular junction

Authors: *A. S. POWERS, G. A. LNENICKA;
Dept. of Biology, SUNY Albany, Albany, NY

Abstract: Repetitive stimulation has been shown to produce various forms of synapse strengthening at the neuromuscular junction (NMJ). Early studies showed that these synaptic enhancements involved an increase in the quantal content of transmitter release and subsequent studies examined the role of presynaptic residual Ca^{2+} in NMJ plasticity. This differs from the CNS where long-term potentiation involves increased postsynaptic glutamate sensitivity and increased quantal current amplitude; these changes are triggered by an elevation in postsynaptic intracellular calcium concentration $[Ca^{2+}]_i$. We have found that the *Drosophila* larval NMJ also shows an activity-dependent increase in quantal current amplitude resulting from greater postsynaptic glutamate sensitivity and this plasticity is elicited by increased postsynaptic $[Ca^{2+}]_i$. Two-electrode voltage clamp was used to record spontaneous miniature excitatory postsynaptic currents (mEPSCs) before and after nerve stimulation. Experiments were performed on muscle fibers 5, 6 and 7 in wandering 3rd instar larvae. For these 3 fibers, nerve stimulation at 20 Hz for 1 minute resulted in a 70 to 150% increase in mEPSC charge transfer and 25 to 30 % increase in mEPSC amplitude . This increase in mEPSC size was expressed about 1 minute after nerve stimulation and persisted for at least 10 minutes. Ca^{2+} has been shown to enter through the postsynaptic glutamate receptors and we found that the increase in quantal currents was dependent upon an increase in postsynaptic $[Ca^{2+}]_i$. BAPTA was injected into muscle fiber 7 to buffer intracellular Ca^{2+}; a lower BAPTA concentration delayed the increase in mEPSC size and

a higher concentration eliminated it.
We iontophoresed glutamate at the NMJ to determine whether the increase in quantal currents was due to greater sensitivity of the postsynaptic membrane to glutamate. Using rapid perfusion, 15 ms glutamate pulses were applied at synaptic boutons on muscle fiber 4 and the pulse amplitude was adjusted to give glutamate evoked excitatory postsynaptic potentials (gEPSPs) of approximately 10 mV. Test pulses were applied at 0.2 Hz then conditioning pulses were delivered at 10 Hz for 1 minute followed again by test pulses. As a result of conditioning, the gEPSP amplitude increased by 70% and there was no increase in postsynaptic membrane resistance. The increase in gEPSP amplitude resulted from an increase in postsynaptic $[Ca^{2+}]_i$ since adding EGTA to the bath blocked the increase in gEPSP amplitude . These results demonstrate an activity-dependent postsynaptic potentiation at the NMJ that has features in common with synapse strengthening seen in the CNS

Disclosures: **A.S. Powers:** None. **G.A. Lnenicka:** None.

Poster

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A Grant-in-Aid for Scientific Research B (26282157)

Title: Kinesthetic illusion induced by pairing of visual and peripheral nerve stimulation causes sustained enhancement of corticospinal tract excitability

Authors: F. KANEKO, R. TAKAHASHI, E. SHIBATA, *Y. ITAGUCHI;
Dept. of Physical Therapy, Sapporo Med. Univ., Sapporo, Japan

Abstract: PURPOSE Associative stimulation, or synchronized input from different pathways, induces long-term potentiation and long-term depression in a manner consistent with Hebbian plasticity. We have reported that kinesthetic illusion induced by visual stimulation (KiNVIS) increases corticospinal tract excitability via association fibers. The present study investigated whether the associative stimuli of KiNVIS and peripheral nerve stimulation (PENS) could induce sustained excitability changes in the corticospinal tract. METHODS Ten healthy young subjects participated in this experiment. The subjects were instructed to be fully relaxed during the

intervention. PENS was applied to the ulnar nerve as the interventional stimulus during KiNVIS. To induce KiNVIS, a movie of index finger movement was presented on the display which covered the subject's actual hand. The spatial position on the display was adjusted until the subject reported feeling as though the on-screen hand was their own. PENS was applied at the point at which the index finger flexed maximally in the movie. PENS was delivered to the right ulnar nerve at the wrist at 300% of the perceptual threshold; however, we altered intensity to 90% of the motor threshold if the perceptual threshold exceeded the motor threshold. The associative stimulation of KiNVIS and PENS was delivered for 15 minutes (3 sets x 5 minutes). Paired-pulse transcranial magnetic stimulation (TMS) and Single-TMS (SgTMS) were used to assess corticospinal excitability and short-interval intracortical inhibition (SICI). We recorded MEP from the right first dorsal interosseous (FDI) muscle and the abductor digiti minimi (ADM) muscle. TMS was applied at the optimal site for eliciting MEP from the right FDI. The intensity of SgTMS and the test intensity of paired-pulse TMS were adjusted to elicit a MEP of approximately 1 mV in amplitude. The conditioning stimulus intensity was 80% of the active motor threshold. The paired-pulse TMS inter-stimulus interval was 2 and 3 ms. MEP was measured twice before the intervention, immediately after the intervention, and 20, 40, 60, and 80 minutes after stimulation. MEP amplitude and SICI were presented as relative values based on before. RESULTS MEP amplitude of the FDI was significantly larger until 60 minutes after stimulation, whereas there were no significant differences in the ADM. There were no significant differences in SICI. CONCLUSIONS These findings indicated that associative stimulation of KiNVIS and PENS can induce a sustained enhancement of corticospinal tract excitability.

Disclosures: F. Kaneko: None. **R. Takahashi:** None. **E. Shibata:** None. **Y. Itaguchi:** None.

Poster

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Program#/Poster#: 504.14/K14

Topic: B.08. Synaptic Plasticity

Title: Depolarization-induced suppression of inhibition in the lateral septum

Authors: *J. M. POWER, K. MOODLEY, A. HARASTA, M. KLUGMANN;
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Abstract: The lateral septum (LS) provides context dependent regulation of mood and motivated behaviour. The majority of neurons in the LS are GABAergic projection neurons that integrate excitatory inputs from hippocampus and send feedforward inhibitory projections to a host of limbic, diencephalic and midbrain regions that control goal-directed, stress, and consumption

behaviours. LS neurons also provide strong feedback inhibition of other LS neurons through extensive local circuit connections. In many brain regions, inhibitory synaptic transmission is transiently suppressed by endocannabinoids released by depolarization-induced calcium influx in post synaptic neurons. Cannabinoid type 1 (CB1) receptors are highly expressed in the LS, however depolarisation-induced suppression of inhibition (DSI) has not been demonstrated in the LS. To resolve this, whole cell patch clamp recordings were made from LS neurons in brain slices prepared from C57Bl/6J mice (4-6 weeks). Pharmacologically isolated inhibitory postsynaptic currents (IPSCs) were readily evoked by a stimulating electrode positioned locally in the LS. Brief depolarization of the post-synaptic neuron (3.5s step from -70 mV to 0 mV) transiently suppressed the IPSC by 18 ± 10% (n=10). DSI was associated with an increase in the paired pulse ratio, indicative of a decrease in the transmitter release probability. No DSI was observed in presence of CB1 cannabinoid receptor antagonists AM251 (4 ± 3%; n=9) or SR141716A (-2 ± 4 %; n=7). These data confirm the existence of cannabinoid-mediated DSI in the LS.

Disclosures: **J.M. Power:** None. **K. Moodley:** None. **A. Harasta:** None. **M. Klugmann:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NRF of Korea-2012R1A3A1050385

Title: Hippocampus-dependent learning and memory deficits by presynaptic dysfunction in LSD1 knock-in mice

Authors: ***C.-S. LIM**[1], J. LEE[1], J. CHOI[1], S. J. KANG[1], S. KIM[1], C. KWAK[1], K.-W. SHIM[1], H. J. NAM[2], J.-H. LEE[3], S. H. BAEK[2], B.-K. KAANG[1];
[1]Biol. Sci., Lab. of Neurobiology, Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Biol. Sci., Lab. of Mol. and Cell. Genetics, Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Life and Nanopharmaceutical Sci., Kyung Hee Univ., Seoul, Korea, Republic of

Abstract: Lysine-specific demethylase 1 (LSD1) is a flavin adenine dinucleotide-dependent histone demethylase and participates in gene repression or activation. Recent studies reported that LSD1 inhibition by chemical inhibitors blocked memory consolidation and LSD1 phosphorylation by PKCα was implicated in circadian rhythmicity independently of its demethylase activity. However, the effect of LSD1 phosphorylation by PKCα on learning and

memory is largely unknown. In this study, we examined the roles of LSD1 in cognition using LSD1 knock-in (KI) mice, in which a PKCα phosphorylation site was mutated. LSD1 KI mice showed partially decreased anxiety and increased locomotion. Interestingly, short-term and long-term contextual fear memory as well as spatial learning and memory in the Morris water maze were impaired in LSD1 KI mice. Extracellular field recordings from Schaffer collateral-CA1 pathways showed that short-term synaptic plasticity such as paired pulse ratio and post-tetanic potentiation was significantly reduced in LSD1 KI mice compared to wild-type littermates whereas long-term synaptic plasticity including long-term potentiation and long-term depression were normal. Moreover, the frequency of miniature EPSC was significantly increased but the amplitude was not changed in LSD1 KI mice, suggesting presynaptic dysfunction in LSD1 KI mice. Consistently, RNA-seq analysis showed that gene expression related to the presynaptic vesicle release was significantly upregulated in LSD1 KI mice. These results suggest that LSD1 plays an important role in hippocampus-dependent learning and memory through presynaptic dysfunction. C.-S. Lim and J. Lee contributed equally to this work.

Disclosures: **C. Lim:** None. **J. Lee:** None. **J. Choi:** None. **S.J. Kang:** None. **S. Kim:** None. **C. Kwak:** None. **K. Shim:** None. **H.J. Nam:** None. **J. Lee:** None. **S.H. Baek:** None. **B. Kaang:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NMSS Grant, PP2321

NIH Grant, HD083727-01A1

Title: Time and intensity determine the effect of downslope walking on soleus but not tibialis anterior H-reflexes

Authors: *M. SABATIER[1], E. ARNOLD[2], T. RIGEL[2], D. LEE[2], B. FARMER[2], M. KEIGHTEY[2], M. HOQUE[2];
[1]Rehabil. Med., [2]Emory Univ., Atlanta, GA

Abstract: We recently found that a 20-min bout of downslope walking (DSW) at 2.5 mph and -15% slope results in acute soleus (Sol) H-reflex depression. The purpose of this study was to determine if 1) DSW-mediated H-reflex depression, previously found only in the Sol, also occurs

in the tibialis anterior (TA), and 2) the effect of DSW on H-reflexes depends on walking time (i.e., duration) or intensity (i.e., slope steepness). Sol and TA H-reflexes were measured before and after DSW at 2.5 mph in fourteen healthy adults on four days. Four DSW time-intensity combinations were used: 10 and 20 mins at -15%, 10 and 20 mins at -25%. Ten participants completed level walking (LW) for 20 mins at 2.5 mph. Sol and TA H-reflex and M-wave recruitment curves were collected while participants maintained EMG background activity at 20% of max. The H-reflex was expressed as H_{max}/M_{max}. Sol H_{max}/M_{max} depression after 10 mins of DSW at -15% was not significantly different from depression after 20 mins of LW (P=0.61), but Sol H_{max}/M_{max} depression after DSW for 20 mins at -15% (-30 ± 21%), 10 mins at -25% (-31 ± 22%), and 20 mins at -25% (-32 ± 23%) was significantly greater than Sol H_{max}/M_{max} depression after both LW for 20 mins (9 ± 15%, $P \leq 0.02$) and DSW for 10 mins at -15% ($P \leq 0.04$). Therefore, more H-reflex depression resulted from DSW than from LW if the duration was at least 20 mins at -15% slope, or if the slope was -25%. Neither LW nor DSW had an effect on TA H-reflexes. Thus, 10 mins of DSW is insufficient, if the slope is -15%, to cause more H-reflex depression than LW for 20 mins, but both 10 and 20 mins at -25% DSW cause significantly more H-reflex depression than LW for 20 mins. However, H-reflex depression found after LW and DSW for the Sol does not extend to the TA. Therefore, DSW-mediated H-reflex depression is muscle-group specific and determined by walking time and intensity. These findings further our understanding of how to tailor DSW as a neuromodulatory methodology to target spinal plasticity, and could guide future efforts in rehabilitation science to induce durable spinal plasticity.

Disclosures: **M. Sabatier:** None. **E. Arnold:** None. **T. Rigel:** None. **D. Lee:** None. **B. Farmer:** None. **M. Keightey:** None. **M. Hoque:** None.

Poster

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Program#/Poster#: 504.17/K17

Topic: B.08. Synaptic Plasticity

Title: Kainate Receptors gating modulates the receptor diffusion at glutamatergic synapses

Authors: ***A. I. POLENGHI**[1], P. GOROSTIZA[2], A. BARBERIS[1];
[1]Fondazione Inst. Italiano Di Tecnologia, Genova, Italy; [2]Inst. of Bioengeneering of Catalonia, Barcelona, Spain

Abstract: The lateral mobility of neurotransmitter receptors is a crucial determinant for the fine tuning of the number of the receptors at the post-synaptic site and, consequently, for the

modulation of synaptic strength. Although a considerable effort has been made to characterize the role of lateral diffusion in the control of neuronal network activity, the relation between the conformational state and the mobility of the receptors is poorly investigated. To address this issue, we manipulated the conformational states of glutamate receptors with light by using Photoswitched Tethered Ligands. In particular, we took advantage of engineered glutamate receptors (LiGluK2Rs) in which a molecular photoswitch operates the glutamate binding and unbinding upon azobenzene *cis/trans* photoisomerization, obtained by illumination with 380 nm and >460 nm light, respectively. By combining the use of LiGluK2 with the single particle tracking technique, we studied the receptor lateral diffusion in controlled LiGluK2 conformational states. We report that transition into desensitization of LiGluK2 receptors markedly and reversibly immobilized receptors at excitatory synapses. In contrast, desensitization did not alter the lateral mobility of extrasynaptic LiGluK2Rs, thus suggesting that this phenomenon is selective for synaptic receptors. Next, we assessed whether the light-induced immobilization of desensitized LiGluK2Rs might be due to the increase of intracellular Ca^{2+} induced by LiGluK2Rs activation. Interestingly, the suppression of the Ca^{2+} permeability of LiGluK2 and the block of the principal sources of intracellular calcium increase did not affect the immobilization of desensitized LiGluK2Rs at synapses. We hypothesized that the trapping of synaptic LiGluK2Rs could be due to altered interactions between scaffold proteins at excitatory synapses and receptors in the desensitized state. To this end, we generated two mutant forms of LiGluK2, lacking of the PDZ binding domain or the β-catenin/N-Cadherin interaction domain. Interestingly, we found that the deletion of the β-catenin/N-Cadherin domain completely abolished the desensitization-induced trapping of LiGluK2 at synapses. To further confirm this result, the use of a dominant-negative form of Delta-N-cadherin prevented the immobilization of synaptic LiGluK2Rs in the desensitized state. We propose here that the conformational changes occurring during transition into desensitization may strengthen the interactions between receptors and specific scaffold proteins, thus leading to the trapping of LiGluK2Rs at excitatory synapses.

Disclosures: **A.I. Polenghi:** None. **P. Gorostiza:** None. **A. Barberis:** None.

Poster

504. Short-Term Plasticity

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Topic: B.08. Synaptic Plasticity

Support: EY014024

Title: Short-term synaptic plasticity within rat thalamocortical circuitry

Authors: *K. R. LOUIS, J. A. BEATTY, C. L. COX;
Michigan State Univ., East Lansing, MI

Abstract: It is well established that information transfer within thalamocortical circuits is dynamically regulated. Considering that tetanic activation of afferent pathways can lead to alterations in synaptic efficacy in many brain regions (i.e., long-term potentiation, long-term depression, and spike-timing dependent plasticity), we carried out a series of experiments to determine if similar forms of plasticity occur in thalamocortical circuits. Whole-cell recordings were obtained from thalamocortical relay neurons in the ventrobasal nucleus (VB). The induction protocol involves tetanic electrical stimulation of corticothalamic afferents (5 pulses at 100Hz, repeated 400 times at 2Hz). This stimulation protocol produced a short-term (10-15 minutes) facilitation of the excitatory postsynaptic current (EPSC) amplitude in 31 of 42 neurons. Using paired-pulse stimulation (100 ms interval), the induction protocol produced a decrease in the paired-pulse ratio, consistent with a presynaptic mechanism. In addition, the potentiation is calcium-dependent, because the enhancement was absent in calcium-free physiological solution. Activation of adenylyl cyclase by forskolin mimicked the potentiation and occluded the subsequent train-induced potentiation indicating the role of the adenylyl cyclase pathway. The adenylyl cyclase inhibitor, SQ22536, significantly reduced the potentiation. Furthermore, the K^+ channel blockers tetraethylammonium chloride (TEA) and 4-aminopyradine (4-AP) attenuated the potentiation, indicating the role of K^+ channels in the potentiation. These data suggest that tetanic activation of corticothalamic afferents activates the adenylyl cyclase pathway, leading to alterations in K^+ conductances that ultimately enhances neurotransmitter release. This potentiation could provide a mechanism through which corticothalamic activity could impact and regulate information processing through thalamocortical circuits.

Disclosures: **K.R. Louis:** None. **J.A. Beatty:** None. **C.L. Cox:** None.

Poster

504. Short-Term Plasticity

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 504.19/L2

Topic: B.08. Synaptic Plasticity

Support: KAKENHI 15K00413

MHLW H27-Kagaku-Ippan-007

Title: Activation of $GABA_A$-receptors by high-frequency stimuli causes paired burst facilitations in area CA1 of the hippocampal slice

Authors: *T. TOMINAGA, Y. TOMINAGA;
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Abstract: The cross-frequency interaction of oscillatory brain activity is thought to have a critical role in animal behavior. In particular, the gamma (25-100 Hz) - theta (4-8 Hz) coupling is considered to have an important computational role in the hippocampus system. During in vitro preparation, a stimulation protocol that mimics the coupled oscillation theta burst stimulation (TBS) is used to induce long-term potentiation (LTP). However, little is known about the mechanism and role of the coupling. Using electrophysiology and voltage-sensitive dye imaging (VSDI), we found that TBS induces the augmentation of spike firing. The augmentation can be induced with only the first pair of TBS, i.e., a pair of short 100 Hz bursts of stimulation intermitted with 170 ms. In this study, we focused on the characteristics of the augmentation caused by a pair of brief bursts of stimulation (paired burst stimulation, or PBS). We found that PBS enhanced membrane potential responses on VSDI signals and intracellular recordings when it was absent in the current recording under whole-cell clamp conditions. The enhancement of the response accompanied the augmentation of the excitatory postsynaptic potential (epsp) to spike firing (E-S) coupling. The paired-burst facilitation (PBF) reaches a plateau when the number of the first burst stimulations (priming burst) exceeds three. Also, the augmentation was seen only after the third and fourth stimulus in the following test burst. Thus, the augmentation always needs a pair of bursts. The interval between the bursts of 150 ms resulted in the maximum PBF. Gabazine (a GABAA receptor antagonist) abolished the PBF. The threshold for the spike generation of the postsynaptic cells measured with a current injection to cells was not lowered by the priming burst of PBS. These results indicate that PBS activates the GABAergic inhibitory system to cause short-term E-S augmentation without raising postsynaptic excitability. The whole cell current of the GABAergic current shows a longer time constant when the stimulation is applied in the bursting form. We propose that a GABAergic inhibitory system of area CA1 of the hippocampus produces the short-term E-S plasticity that could cause exaggerated spike-firing upon a theta-gamma activity distinctively, thus making the neural circuit of the CA1 act as a specific transmitter of the specific oscillation signal.

Disclosures: **T. Tominaga:** None. **Y. Tominaga:** None.

Poster

504. Short-Term Plasticity

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 504.20/L3

Topic: B.08. Synaptic Plasticity

Support: NSERC

Title: Influence of single session aerobic exercise on trafficking of receptors involved in neuroplasticity

Authors: *J. S. THACKER[1], W. R. STAINES[1], J. G. MIELKE[2];
[1]Kinesiology, [2]Sch. of Publ. Hlth. and Hlth. Systems, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: The priming of motor cortex to facilitate plasticity is an approach that may improve motor rehabilitation following injury. Emerging evidence supports the possibility that aerobic exercise may offer one means to achieve priming, however, the mechanisms whereby priming may occur following an acute bout of aerobic exercise are not clear. Although one popular hypothesis stems from data displaying robust increases in neurotransmitter and neurotrophic factor release in brain during and after exercise, this phenomenon appears broadly, and may not alone explain changes occurring within specific regions, such as motor cortex. An alternate explanation may be related to the migration of receptors towards synaptic densities, which has been shown to play a key role in plasticity. Possibly, aerobic exercise may prime neuroplasticity through both increased neurotransmitter/neurotrophic release and the reorganization of the synaptic receptor population, and we sought to investigate the latter option. Specifically, we hypothesized that a single session of exercise would promote the trafficking of plasticity related receptors (AMPA receptor/NMDA receptor/TrkB; BDNF receptor) to synaptic terminals, and that this change in their cellular distribution would act as a molecular mechanism for priming of motor cortex. Young, male Sprague-Dawley rats (n = 18) on a reverse light cycle (12h/12h) were exposed to a gradual treadmill acclimatization procedure lasting 8 days (10 m/min up to 25 m/min) for 10 minutes at the same time (10 am) each day. Acclimatization was followed by 2 days of rest to reduce any carryover effects. On testing day, rats were separated into either exercise (exhaustion/steady state moderate), or non-exercising groups. Immediately following exercise, rats were sacrificed and tissue slices prepared from both the motor cortex and hippocampus. Surface proteins were labelled by incubating slices in biotin. After homogenization, synaptoneurosomes were prepared (to enrich pre/post-synaptic terminals), and then proteins at the synaptic membrane were isolated by incubation with neutravidin beads. We plan to probe all surface synaptic fractions for proteins of interest by Western blotting analysis. The work in progress aims to establish that a single session of aerobic exercise is sufficient to promote a “primed” state within motor cortex by influencing the trafficking of a particular set of plasticity-related receptors.

Disclosures: **J.S. Thacker:** None. **W.R. Staines:** None. **J.G. Mielke:** None.

Poster

505. Structural Plasticity II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 505.01/L4

Topic: B.08. Synaptic Plasticity

Support: NSF IOS-1526941

CRCNS RO1MH102841-03

U01NS090583-02

NIH R56NS096710-01

Title: Synapse labelling by PSD95FingR-GFP intrabody does not affect LTP induced by glutamate uncaging in single spines

Authors: *N. OTMAKHOV, J. LISMAN;
Biol. Department, Volen CCS, Brandeis Univ., Waltham, MA

Abstract: Labeling of synaptic proteins by fluorescent expressible proteins is a valuable tool for investigation of synapse growth during synaptic plasticity in real time in living cells. Previous work, however, showed that overexpression of synaptic proteins such as PSD95 fused with fluorescent proteins (GFP) can by itself produce synapse/spine growth, a growth that can preclude further induction of LTP. Recently a new approach was developed for labelling PSD95 by antibody-like expressible proteins such as PSD95FingR-GFP intrabodies (Gross et al., 2013). This approach was shown to accurately mark localization of the endogenous PSD95 without altering spine density or the amplitude/frequency of mEPSPs. What has not been tested is whether this type of labeling alters synaptic plasticity processes such as LTP. Here we expressed PSD95FingR-GFP intrabody together with mCherry (as cell volume marker) in hippocampal pyramidal CA1 neurons in slice cultures. Structural LTP (sLTP) in single dendritic spines was induced by 2-photon glutamate uncaging and estimated by a sustained increase in spine volume measured by two-photon imaging of mCherry. As a control, single spine sLTP in neurons expressing untagged GFP was measured in interleaved experiments. PSD95FingR-GFP intrabody expression was strongly enriched in spines. Glutamate uncaging produced significant initial spine growth which was stabilized at steady state level of 180% at 60 min after induction ($n = 20$) which was not significantly different from sLTP induced in GFP labelled cells ($n = 5$, $P > 0.05$). Interestingly, total PSD95FingR-GFP fluorescence in spines transiently increased (40% for < 15 min) with the initial phase of spine growth and remained slightly above the baseline during the steady state phase of LTP. The calculated bound pool of the PSD95FingR-GFP, however, transiently decreased (20% for < 10min) after uncaging and remained at the baseline

for at least 60 min after sLTP induction. We conclude that synaptic labelling by PSD95FingR-GFP intrabody does not alter sLTP for at least 60 min. Currently, we exploring a possibility to use PSD95FingR-GFP labelling for measuring synapse growth during late phase of sLTP.

Disclosures: N. Otmakhov: None. **J. Lisman:** None.

Poster

505. Structural Plasticity II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 505.02/L5

Topic: B.08. Synaptic Plasticity

Support: Paris Sciences et Lettres

Labex Memolife

Title: Measuring cannabinoid-induced presynaptic structural plasticity by 3D 2-color STORM nanoscopy

Authors: M. H. MCFADDEN[1], C. LETERRIER[2], *D. ZALA[1], Z. LENKEI[1];
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Abstract: The endocannabinoid system is an important regulator of functional plasticity within the mammalian brain and has now long been associated to both long and short term forms of synaptic plasticity, both essential for learning and memory. Recent findings have suggested, using electron microscopy, that this functional plasticity may be a direct effect of structural reorganization of the presynaptic compartment following activation of the cannabinoid type 1 receptor (CB1R), the main cannabinoid receptor in the mammalian brain. Given these findings, we are currently investigating the structural relationship between the different presynaptic elements that may be affected by CB1R activation. As presynaptic boutons are relatively minute in size, measuring on average about 1μm^3, the structural changes expected to occur under CB1R activation are on the nanometer scale. For this reason, we have chosen to resort to STORM nanoscopy, one of the most resolved microscopy techniques currently available. Using 3D 2-color STORM and immunostaining for different vesicle markers and synaptic scaffolding proteins, we are now able to measure the nanoscale relationship between synaptic vesicles, the presynaptic active zone and the postsynaptic scaffold and observe cannabinoid-induced nanoscale reorganization of these elements. Furthermore, given the computationally intensive nature of STORM localization measurements, we are developing a user-friendly technique for

rapidly measuring spatial relationships within chosen ROIs of 3D 2-color STORM images by integrating currently available STORM data processing ImageJ plugins.

Disclosures: **M.H. McFadden:** None. **C. Leterrier:** None. **D. Zala:** None. **Z. Lenkei:** None.

Poster

505. Structural Plasticity II

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Topic: B.08. Synaptic Plasticity

Support: NSERC Grant 249853

Title: Using the lipophilic dye DiI to study dendritic spine alterations in hippocampal slices

Authors: *J. S. TRIVINO PAREDES[1], P. C. NAHIRNEY[1,2,3], B. R. CHRISTIE[1,2,3];
[1]Div. of Med. Sci., [2]Dept. of Biol., Univ. of Victoria, Victoria, BC, Canada; [3]Dept. of Cell. and Physiological Sci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The hippocampus is a key brain structure involved in learning and memory, and has been used as a model structure for studying synaptic plasticity, a biological model for learning and memory processes. In many studies, hippocampal slices are widely used for *in vitro* electrophysiological experiments to study the cellular mechanisms involved in synaptic transmission and plasticity. To optimize slice viability and hopefully recapitulate *in vivo* physiological conditions *in vitro*, a number of variables, including: age of the animal; composition of the artificial cerebrospinal fluid (aCSF); temperature; and slice cutting methodology are taken into consideration. However, previous studies using electron microscopy and two-photon microscopy approaches have reported that dendritic spines, neuronal structures crucial to the process of excitatory synaptic transmission, are dramatically altered as compared to those normally observed in perfusion-fixed slices. These alterations in dendritic spines could represent a serious confound for *ex vivo* work, and lead to the results that vary markedly from *in vivo* electrophysiological experiments. In these experiments, we employed the lipophilic carbocyanine dye 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (DiI) in combination with confocal microscopy to compare dendritic spine density in the *Cornu Ammonis 1* and *Dentate Gyrus* areas in perfusion-fixed slices and slices prepared for *in vitro* recordings. We found that in both young and adult Sprague-Dawley rats there was an increase in dendritic spine density in slices prepared for *in vitro* recordings. These results in conjunction with observations in previous studies highlights the fact that slicing-induced spinogenesis is an important factor to take into consideration when studying functional and structural plasticity.

Disclosures: **J.S. Trivino Paredes:** None. **P.C. Nahirney:** None. **B.R. Christie:** None.

Poster

505. Structural Plasticity II

Location: Halls B-H

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Program#/Poster#: 505.04/L7

Topic: B.08. Synaptic Plasticity

Support: NIH R01MH106489

Title: The k63 linkage-specific deubiquitinase cyld regulates synapse formation, remodeling, and plasticity

Authors: ***E. M. ZAJICEK**, Q. MA, H. RUAN, W. BURNETTE, W.-D. YAO;
SUNY Upstate Med. Univ., Syracuse, NY

Abstract: Lysine-63 (K63) linked ubiquitin chains are primarily non-proteasomal, and their exact role is not well understood in the mammalian brain. However, they are known to play a key role in various immune signaling functions and diseases. CYLD is a K63-linkage-specific deubiquitinase that has been extensively studied in the immune system for its role in NFκB signaling and the development of cylindromatosis (turban tumor syndrome) in humans. Recent evidence, however, has shown that CYLD is abundant in the brain, especially in the postsynaptic density (PSD) of excitatory synapses, and is recruited to the PSD following neuronal activity. We propose that K63 ubiquitin chains, as well as their ubiquitin and deubiquitinating enzymes, play a role in the regulation of synaptic development, maintenance, and plasticity. To test this hypothesis, we employed a series of molecular, behavioral, and electrophysiology experiments. Preliminary results indicate that CYLD plays an important role in the regulation of synaptic receptor subunits and morphological remodeling of dendritic spines in cultured hippocampal neurons. Preliminary experiments also suggested alterations in learning, memory, reward and other behaviors in mice lacking CYLD. Further work will be done to confirm the functional role of CYLD and K63 linked ubiquitin chains and to identify the mechanism of action.

Disclosures: **E.M. Zajicek:** None. **Q. Ma:** None. **H. Ruan:** None. **W. Burnette:** None. **W. Yao:** None.

Poster

505. Structural Plasticity II

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Program#/Poster#: 505.05/L8

Topic: B.08. Synaptic Plasticity

Support: NIH/NINDS NS027177-27 (Tsien)

NIH/NIGMS grant 103412 (Ellisman)

Title: Are very long-term memories stored in the pattern of holes in the perineuronal net?

Authors: *V. LEV-RAM[1], E. A. BUSHON[2], T. J. DEERINCK[2], K. M. TALIMAN[3], D. R. PRITCHARD[3], A. PEREZ[2], D. B. MCCLATCHY[5], J. R. SAVAS[6], J. R. YATES, third[5], M. H. ELLISMAN[2], R. Y. TSIEN[4];
[1]UCSD Sch. Med., La Jolla, CA; [2]Neurosci., [3]Pharmacol., [4]Pharmacology, Chemistry, HHMI,, UCSD, San Diego, CA; [5]The Scripps Res. Inst., San Diego, CA; [6]Northwestern Univ., Chicago, IL

Abstract: The PNN is a specialized form of extracellular matrix, initially deposited around selected neurons during critical periods of development in specific parts of the brain, interrupted by holes where synapses occur. We postulate that the PNN comprises a longer-lived structural template and that new memories are created by cutting new holes in the PNN or by expanding existing holes to enable formation of new synapses or to strengthen existing ones. A basic premise of this hypothesis is that the PNN, should undergo very low metabolic renewal from the first age at which memories are retained until senescence, whereas the active constituents of synapses turn over much more frequently and would therefore be poorer substrates for permanent information storage, unless they are equipped with incredibly accurate copying mechanisms (R.Y.Tsien PNAS 2013). We continue to examine this hypothesis using several experimental approaches: 1. Using ^{15}N Spirulina diet for Stable Isotope Labeling in Mammals (SILAM) we compare the lifetimes of PNN proteins vs. intra-synaptic components in Enriched Environment (EE) vs. Conventional Cages (CC), ending the pulse-case by changing to ^{14}N diet at P45 vs. P21, in four different brain areas. Analysis by Multidimensional Protein Identification Technology (MudPIT) indicate: a. Low turnover rate for PNN proteins while synaptic proteins were at the noise level of $^{15}N/^{14}N$ ratio. b. Higher turnover of PNN proteins in EE vs. CC cages c. Lower $^{15}N/^{14}N$ ratio when the pulse chase was terminated at P21 vs. P45 (i.e. before vs. after the closure of many critical periods). d. Variability in the retention of ^{15}N in PNN proteins between brain areas. 2. 2D EM and 3D volumes of Serial Block Face Scanning EM reveal that neurons engulfed in PNN labeled with WFA have more than 95% of their plasma membrane surface occupied by PNN or synapses. 3. The role and timing of matrix metalloproteinase (MMP) activity in memory consolidation using pharmacological inhibitors in a fear-conditioning

paradigm. Our results demonstrate that MMP inhibition during fear conditioning training: a. Does not affect acquisition. b. Significantly impairs long-term memory (30 days). c. Is dose dependent. d. Memory impairment increases with time. 4. Mapping PNN location in mouse brain tissues to test correlation between regions that are known to take a role in very-long-term memory and high density of PNN engulfed neurons.

Disclosures: **V. Lev-Ram:** None. **E.A. Bushon:** None. **T.J. Deerinck:** None. **K.M. Taliman:** None. **D.R. Pritchard:** None. **A. Perez:** None. **D.B. McClatchy:** None. **J.R. Savas:** None. **J.R. Yates:** None. **M.H. Ellisman:** None. **R.Y. Tsien:** None.

Poster

505. Structural Plasticity II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 505.06/L9

Topic: B.08. Synaptic Plasticity

Support: Boehringer Ingelheim/Ulm University BioCenter (BIU)

Title: sAPPα and sAPPβ increase structural complexity and synapse generation in primary hippocampal neurons by altering Ca^{2+} homeostasis

Authors: ***R. HESSE**[1], D. FERNÁNDEZ-FERNÁNDEZ[2], K. J. FOEHR[3], K. KROKER[2], D. SCHWANZAR[1], H. ROSENBROCK[2], C. A. F. VON ARNIM[1];
[1]Ulm University/Neurology, Ulm, Germany; [2]Boehringer Ingelheim GmbH & Co KG, Dept. of CNS Dis. Res., Biberach, Germany; [3]Ulm University/Dept. of Anesthesiol., Ulm, Germany

Abstract: Amyloid-β (Aβ) generation from its precursor (APP) is either prevented or promoted, depending on the APP cleavage pathway. In Alzheimer's disease (AD) patients APP cleavage is shifted towards the amyloidogenic pathway. Aβ is known to have severe impact on synaptic function, however much less is known about the physiological functions of APP. The neurotrophic properties of sAPPα are well-established, whereas only few and conflicting studies on sAPPβ function exist. The cellular mechanisms of sAPP functions are largely unknown. Since the main domains involved in neurotrophic functions of sAPPα are also present in sAPPβ we performed a head-to-head comparison of both mammalian recombinant peptides in primary hippocampal neurons (PHN). Binding assays revealed a co-localisation of exogenous applied sAPP with MAP-2 in PHN. We found that sAPPα significantly increase axonlength (p=0.0002) and that sAPPα and sAPPβ increase neurite number (p<0.0001) of PHN at DIV7 but not at DIV4. Moreover both sAPPα and sAPPβ treated neurons showed a higher neuritic complexity in Sholl analysis. Number of glutamatergic synapses (p<0.0001), as well as layer thickness of

PSDs, are significantly increased upon sAPP overexpression in PHN. Although sAPP pre-treatment of PHN elevated glutamate sensitivity of PHN (p<0.05) analysed by Calcium-Imaging, glutamate-induced currents are not affected by sAPP overexpression. Taken together our results indicate that sAPPα as well as sAPPβ have critical stage-dependent roles in neuronal development by increasing glutamate sensitivity, but do not alter synaptic activity.

Disclosures: R. Hesse: None. **D. Fernández-Fernández:** None. **K.J. Foehr:** None. **K. Kroker:** None. **D. Schwanzar:** None. **H. Rosenbrock:** None. **C.A.F. von Arnim:** None.

Poster

505. Structural Plasticity II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 505.07/L10

Topic: B.08. Synaptic Plasticity

Title: Rapid postsynaptic cAMP signaling regulates structural and functional potentiation underlying learning and memory

Authors: *T. LUYBEN[1,2], J. BOROVAC[2], M. VALENCIA[2], M. KHAN[1], T. TOMINAGA[3], K. OKAMOTO[1,2];
[1]SLRI, Samuel Lunenfeld Res. Inst., Toronto, ON, Canada; [2]Mol. Genet., Univ. of Toronto, Toronto, ON, Canada; [3]Inst. of Neurosci., Tokushima Bunri Univ., Sanuki, Japan

Abstract: Functional and structural modifications of dendritic spines are tightly correlated and play an essential role in synaptic plasticity. Postsynaptic cAMP signaling is crucial for an enhancement of synaptic function such as the protein synthesis-dependent late phase of long-term potentiation (L-LTP). However, the role of cAMP in the structural change of synapses remains elusive. Here we provide evidence for a rapid postsynaptic cAMP/PKA function in structural potentiation of dendritic spines (sLTP) without protein synthesis signaling. By monitoring postsynaptic cAMP dynamics during LTP using two-photon FRET microscopy, we detected NMDA receptor-dependent transient cAMP production in dendritic spines and the dendrite during L-LTP induction, but not the early-phase (E-LTP). This demonstrated the presence of postsynaptic cAMP signaling in L-LTP but not E-LTP. Next, to address the function of cAMP in structural plasticity, we induced L-LTP by tetanic stimulation and generated sLTP at a neighbouring unaffected spine on the same neuron. We found a prolonged spine enlargement during sLTP following neighboring synapse L-LTP induction that could be blocked by a PKA inhibitor, suggesting a role for cAMP in sLTP. We also mimicked cAMP production by two-photon photoactivation of photoactivatable adenylyl cyclase (PAC) at single spines, and found the cAMP/PKA function in sLTP did not require either new protein synthesis or

hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. It was necessary to produce cAMP within a 1 min time window after sLTP induction, suggesting rapid cAMP signaling is involved in the regulation of sLTP. In addition to the structural function, we also found that the rapid postsynaptic cAMP enhanced synaptic function after LTP induction by employing voltage-sensitive dye (VSD) imaging and field recording methods. We will discuss rapid and new protein synthesis independent cAMP function and mechanism in structural and functional plasticity underlying learning and memory.

Disclosures: **T. Luyben:** None. **J. Borovac:** None. **M. Valencia:** None. **M. Khan:** None. **T. Tominaga:** None. **K. Okamoto:** None.

Poster

505. Structural Plasticity II

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Program#/Poster#: 505.08/L11

Topic: B.08. Synaptic Plasticity

Support: NIH F32 MH 101954

Max Planck Florida

Title: Imaging the activity of classic protein kinase C isozymes during single spine structural plasticity

Authors: *L. A. COLGAN, P. PARRA-BUENO, R. YASUDA;
Max Planck Florida Inst., Jupiter, FL

Abstract: Synaptic plasticity is mediated by complex signaling cascades that transduce short-lived calcium inputs into long-lasting changes of synaptic strength. Classic protein kinase C (PKC) isozymes, which are activated by both calcium and lipid signaling to phosphorylate downstream targets, are well suited to play a role in this process. However, limitations in current approaches have impeded our understanding of the specific role of these PKC isozymes in plasticity. To determine whether classic PKC isozymes are required for plasticity, isozyme-specific PKC knockout animals were screened for plasticity deficits. Only disruption of PKCa function resulted in structural plasticity deficits which could be rescued by expression of PKCa, but not by other classic isozymes PKCb or PKCg, suggesting specificity of isozyme signaling even amongst these closely related isozymes. To further study the role of PKCa signaling in plasticity, we developed optical sensors to measure the isozyme-specific activity of classic PKCs during the induction of plasticity in single spines. In response to glutamate release PKCa was

robustly activated in a rapid and transient manner, with kinetics that differed from PKCb and PKCg activity. With each release of glutamate, PKCa translocated to the membrane and bound substrate. Maximal activation required both NMDAR activation as activation of PLC lipid signals, suggesting that PKCa serves as a coincidence detector of these canonical plasticity pathways. Consistent with these findings, PKCa KO animals show impaired long-term potentiation and deficits in spatial learning.

Disclosures: L.A. Colgan: None. **P. Parra-Bueno:** None. **R. Yasuda:** None.

Poster

505. Structural Plasticity II

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Program#/Poster#: 505.09/L12

Topic: B.08. Synaptic Plasticity

Support: STARS Award

Mitchell Center for Neurodegenerative Diseases

UT Start-Up Funds

Title: A novel *Drosophila* protein regulates apposition of the active zones and postsynaptic densities.

Authors: *A. M. BUCKLEY-SHAW, R. NATARAJAN, Y. WAIRKAR;
Neurol., Univ. of Texas Med. Br., Galveston, TX

Abstract: The synapse is the fundamental link for communication amongst neurons. Formation and function of neural circuitry, learning, as well as storage and retrieval of memories are all dependent on transfer of information via the synapses. Indeed, synaptic dysfunction may underlie many of the neurodevelopmental and neurodegenerative disorders, including Autism Spectrum disorder (ASD) and Alzheimer's disease. One of the key aspects of efficacious synaptic transmission is the apposition of presynaptic active zones with the postsynaptic densities. Therefore, understanding how normal apposition of the presynaptic active zones and the postsynaptic densities is crucial to understand how fast neurotransmission at the synapse is accomplished. However, very few molecules are known that regulate the apposition of active zones and postsynaptic densities. To seek more molecules and to thereby, better understand the process of synaptic apposition, we performed a large-scale screen at the *Drosophila* NMJ synapses. Using the marker for active zones (BRP) and postsynaptic densities (DGLURIII), we

screened a collection of P-element mutations and found one gene that showed a change in the apposition of synapses. Our preliminary data with this novel gene will be presented.

Disclosures: **A.M. Buckley-Shaw:** None. **R. Natarajan:** None. **Y. Wairkar:** None.

Poster

505. Structural Plasticity II

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Topic: B.08. Synaptic Plasticity

Support: Brain Research Foundation Scientific Innovations Award

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Human Frontier Science Program

Title: Ultrastructural examination of synapses following channelrhodopsin2-induced long-term potentiation

Authors: ***M. KUWAJIMA**[1], G. CAO[1], B. L. KAJS[1], K. M. HARRIS[2], B. V. ZEMELMAN[2]; [1]Ctr. for Learning & Memory, [2]Ctr. for Learning & Memory, Dept Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: A longstanding question in neuroscience concerns the cellular mechanisms of learning and memory. Addressing this question requires substantial improvement in our ability to identify the synapses involved. It is then possible to examine the functional, cellular, and molecular processes that have been engaged by learning or cellular models of learning such as long-term potentiation (LTP). Unequivocal detection of changes in synapses and their subcellular components requires (1) confidence that specific synapses have been potentiated, and (2) their three-dimensional reconstruction from serial section electron microscopy (3DEM). We re-engineered a genetically encoded EM tag based on the plant ascorbate peroxidase (APEX2; Lam et al., 2015, Nat Methods 12:51-4) to optimize its expression and targeting in neurons, leading to enhanced contrast for 3DEM. (We refer to our re-engineered enzyme as mAPEX2.) An adeno-associated virus (AAV) vector was constructed to co-express mAPEX2 with Chr2-EGFP

(channelrhodopsin2, ET/TC variant) as two proteins separated by the self-cleaving 2A peptide (P2A) from porcine teschovirus. The virus was injected unilaterally into the hippocampal area CA3 of adult mice. One group of AAV-injected mice was used to test mAPEX2 function. After perfusion-fixation with 2.5% glutaraldehyde and 2% formaldehyde, AAV-infected neurons were visualized successfully with nickel-enhanced diaminobenzidine (Ni-DAB), which resulted in electron-dense deposits in mAPEX2-expressing axons. Their visualization through serial section electron micrographs reveal well-preserved tissue for 3DEM analyses. In another group of animals, LTP was induced at CA3 → CA1 synapses in acute hippocampal slices using high-frequency light pulses. Our light stimulation protocol consisted of 3 trains of 100 pulses at 50 Hz, with each pulse lasting 1 ms and 15 s intervals between 100-pulse trains (λ = 473 nm at ~13.5 mW). This was sufficient to induce LTP lasting for 2 hrs, as measured by changes in the slope of field excitatory postsynaptic potential. At end of recording, the slices were chemically fixed (enhanced by a brief microwave irradiation), then stained with (Ni-DAB) to visualize the mAPEX2-expressing axons in the area CA1. 3DEM analysis of the labeled axons and synapses is currently underway. Our new approach will greatly facilitate the detection of synapse-specific ultrastructural changes associated with long-term synaptic plasticity, a crucial first step in elucidating the cellular mechanisms of learning and memory.

Disclosures: **M. Kuwajima:** None. **G. Cao:** None. **B.L. Kajs:** None. **K.M. Harris:** None. **B.V. Zemelman:** None.

Poster

505. Structural Plasticity II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 505.11/L14

Topic: B.08. Synaptic Plasticity

Support: NIH NS062736

Whitehall 2014-05-99

Title: Saturation of structural plasticity at individual dendritic spines

Authors: *O. VIVAS[1], W. C. OH[2], L. PARAJULI[2], K. ZITO[1];
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Abstract: Long-term potentiation (LTP) of synaptic strength is widely accepted as one of the key cellular modifications that support learning. One interesting property of LTP is that it saturates and that saturation impairs learning. In this project, we are interested in understanding

the saturation of structural and functional plasticity at the level of single dendritic spines. Spines are tiny dendritic protrusions (<0.1 μm^3) that serve as the site of most excitatory synapses in the cerebral cortex. Spine morphology is tightly coupled to spine function: spine volume is highly correlated with the area of the postsynaptic density, the number of vesicles in the presynaptic membrane, the number of AMPA receptors, and the amplitude of excitatory postsynaptic currents. Notably, individual dendritic spines exhibit structural and functional plasticity in response to local glutamatergic stimulation. To study the saturation of structural plasticity, we used two-photon glutamate uncaging to examine the parameters that limit the repetitive induction of LTP at individual dendritic spines of hippocampal CA1 neurons. We also are investigating if spines grow in small increments and if there is a predetermined size volume reached for each spine, beyond which spines do not grow. Indeed, as spines grow in response to LTP stimuli, we found that it is increasingly unlikely that the same uncaging protocol will induce additional growth. We are currently studying the molecular mechanisms that place the upper limit on spine size.

Disclosures: **O. Vivas:** None. **W.C. Oh:** None. **L. Parajuli:** None. **K. Zito:** None.

Poster

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Title: Maturation of sensory networks through homeostatic structural plasticity

Authors: ***J. GALLINARO**, S. ROTTER;
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Abstract: Neurons in the adult visual cortex of mice prefer to make synapses with neurons responding to similar visual features. As such a bias in connectivity is not observed at the time of eye opening, it has been proposed that the functional subnetworks are formed through rewiring of recurrent synaptic connections, induced by visual experience [1]. However, it is not clear according to which rules this structure develops. The emergence of feature specific wiring was recently demonstrated in a balanced network model with appropriate rules of functional synaptic

plasticity [2]. In this model, however, connectivity was evaluated based on the strength of already existing synapses, and the structure of the network remained unchanged throughout the simulation.
Referring to recent findings of homeostatic regulation of cortical activity in rodent visual cortex *in vivo* [3,4], we employ here a structural plasticity rule based on firing rate homeostasis described previously [5] for simulating network restructuring during sensory stimulation. We show that, next to other biologically meaningful properties, feature specific connectivity also emerges in a balanced network of changing structure, using a plasticity rule that does not depend on spike timing.
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[5] van Ooyen A. Using theoretical models to analyse neural development. *Nature Reviews Neuroscience* 12: 311-326, 2011

Disclosures: J. Gallinaro: None. **S. Rotter:** None.

Poster

505. Structural Plasticity II

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Topic: B.08. Synaptic Plasticity

Support: Swedish Research Council

The Kamprad Family Foundation

Title: Dopamine D2 receptors are related to physical fitness and exercise: Evidence from an exercise intervention on older adults

Authors: *L. S. JONASSON[1,2], K. RIKLUND[3,2], A. F. KRAMER[4], L. NYBERG[5,2,3], C.-J. BORAXBEKK[6,2];
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Sci., Umeå University, Sweden; [4]Beckman Inst., University of Illinois, IL; [5]Dept. of Integrative Med. Biol., Umeå University, Sweden; [6]Ctr. for Aging and Demographic Res., Umeå University, Sweden

Abstract: In the PHysical Influences on BRain in Aging (PHIBRA) study, 60 sedentary elderly individuals (64-78y) completed a randomized physical activity intervention for 6 months, contrasting aerobic exercise with a stretching and toning active control condition. For the first time on human subjects, the relation between physical fitness and *dopamine D_2 receptors* (D2R) was investigated. D2R availability was measured with *positron emission tomography* (PET) and [^{11}C]raclopride before and after the intervention. We focused on striatum, a subcortical structure serving several important motor and cognitive functions. At baseline, higher aerobic capacity (peak oxygen uptake) was associated with higher D2R availability in striatum. If D2R is assumed to decrease with age, one explanation for the cross-sectional analysis is that the aging related reduction of D2R is slowed by maintaining a higher level of physical fitness at an older age. Following training improved aerobic capacity was related to a change in D2R. Specifically increased peak oxygen uptake was related to an increased D2R in left sensorimotor striatum (k = 16, mni coordinates [-30 -15 -2]), and reduced D2R in left limbic and associative striatum (k = 59, mni coordinates [-9 9 4]). The reduced D2R in anterior regions potentially reflects an increase in endogenous dopamine following improved aerobic capacity since increased endogenous dopamine may lead to a lower estimation of D2R availability. The increased D2R in relation to improved aerobic capacity likely reflects, similar to the findings at baseline, either an increase in D2R, or a slowed reduction in D2R. This is the first study to show 1) a relation between physical fitness and the human dopamine D2R, and 2) that human D2R is sensitive to change in aerobic capacity, which is likely one reason as to why staying physically fit reduces the risk of age-related decline in brain health.

Disclosures: L.S. Jonasson: None. **K. Riklund:** None. **A.F. Kramer:** None. **L. Nyberg:** None. **C. Boraxbekk:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH DC 011137

Title: Mitral cell dendritic arbors recover morphology after chronic deafferentation in the adult zebrafish olfactory bulb

Authors: *J. M. DICKENS, C. A. BYRD-JACOBS;
Western Michigan Univ., Kalamazoo, MI

Abstract: The dendritic arbors and processes of neurons are complex structures, and their morphologies are critical for proper cellular function. Synaptic connections are essential for development and maintenance of dendritic shape. Using mitral cells, the primary output neurons of the olfactory bulb, we examined the afferent/target interactions necessary for the maintenance of dendritic morphology and the potential for the recovery of these structures following long-term removal of sensory input in a reversible deafferentation model. Disruption of afferent input through repeated chemical ablation of the olfactory organ with detergent results in a significant decrease in total number of major dendritic branches, reduction in length of the branches, and diminished area of the dendritic tuft following eight weeks of chronic treatment. There is also a significant decrease in overall dendritic complexity and prevention of growth-related elaboration of the dendritic arbors. The hypothesis of this study was that the size and complexity of mitral cell dendritic arbors would return after recovery of afferent innervation following long-term deafferentation. The olfactory epithelium of adult male and female zebrafish was chemically ablated with the detergent Triton X-100 every three days for eight weeks, followed by cessation of treatment and recovery for a further three or eight weeks. Mitral cells were labeled using retrograde tract tracing with a fluorescent dextran injected into the olfactory tracts of isolated whole brains in culture and imaged using whole-mount confocal microscopy. Projection images of unidendritic mitral cells from unoperated external control fish and detergent-treated fish allowed a recovery period were examined, and the total number of major dendritic branches, length of major dendritic branches, and area of the dendritic arbor were analyzed. A modified Sholl analysis was used to examine the changes in overall dendritic complexity. Following eight weeks of recovery, there were no significant differences in the number of major dendritic branches ($p>0.05$) or the length of those major branches ($p>0.05$) compared to mitral cells of control fish. Additionally, compared to untreated control cells there was no significant difference in the area of the dendritic tuft ($p<0.05$) or in the overall complexity of the dendritic arbor ($p<0.05$). Thus, eight weeks of recovery following chronic deafferentation allows mitral cell dendrites to return to control morphologies. These results provide us with a model system that allows investigation of the regenerative capabilities of adult brain structures.

Disclosures: J.M. Dickens: None. **C.A. Byrd-Jacobs:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NSF Grant 0845285

NARSAD Grant 23312

Title: The role of structural versus functional potentiation in the stabilization of nascent dendritic spines

Authors: *J. T. LAMBERT, T. C. HILL, K. ZITO;
Univ. of California Davis, Davis, CA

Abstract: Animals have a remarkable capacity to change their behavior to suit a wide range of circumstances. At the cellular level, this behavioral flexibility is made possible because individual neurons modify their connectivity in response to varying patterns of synaptic activity in a process termed experience-dependent plasticity. During experience-dependent plasticity, the selective stabilization of new dendritic spines is linked tightly with functional changes in neural circuits. We have shown previously that high-frequency stimulation that induces long-term potentiation (LTP) increases the stability of new spines. Here, we further investigate the cellular and molecular mechanisms of this activity-dependent structural stabilization. We found that stabilization-inducing activity at new spines increases the enrichment of SAP97, a synaptic scaffolding molecule that binds and clusters AMPARs. High-frequency stimulation at individual spines activates pathways for both functional LTP, through the recruitment of additional AMPARs, and structural LTP, through regulation of the actin cytoskeleton. Our finding that a protein important for recruiting AMPARs accumulates at new spines following an LTP stimulus that induces new spine stabilization raised the questions: Is recruitment of AMPARs to new spines necessary for stabilization and, if so, are AMPARs playing a functional or structural role? We will present the results of our ongoing studies to define the relative role of structural versus functional potentiation in the stabilization of nascent spiny synapses.

Disclosures: J.T. Lambert: None. **T.C. Hill:** None. **K. Zito:** None.

Poster

505. Structural Plasticity II

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Topic: B.08. Synaptic Plasticity

Support: CIHR

Title: Glial TNF regulates dendritic spine density in the nucleus accumbens

Authors: *S. C. KONEFAL, J.-P. CLÉMENT, S. CHIERZI, K. MURAI, D. STELLWAGEN; Ctr. for Res. in Neurosci., McGill Univ., Montreal, QC, Canada

Abstract: The nucleus accumbens (NAc) plays an important role in generating motivated behaviors to natural rewards and drugs of abuse. We have previously reported that tumor necrosis factor alpha (TNFα) decreases excitatory synaptic strength in the NAc core following repeated cocaine administration via the endocytosis of AMPA-type glutamate receptors on medium spiny neurons (MSNs). TNFα is produced by glia in the brain, the two main types of which are microglia and astrocytes. Both cell types have been shown to regulate the structural plasticity of dendritic spines on neurons. We propose that glia-derived TNFα regulates the formation and maintenance of dendritic spines on MSNs and plays a role in the cocaine-induced changes in spine density. Repeated exposure to cocaine followed by a period of withdrawal produces an enduring increase in dendritic spine density. We report that adult TNFα -/- mice have an increased baseline spine density in MSNs and, following prolonged withdrawal from cocaine, also show an increase in spinogenesis as compared to wild-type mice. This result suggests a regulatory role for TNFα in dendritic spine formation or maintenance. Our preliminary data further suggest that TNFα derived from astrocytes and microglia have different roles regulating spine density where astrocytic TNFα controls baseline spine density and microglial TNFα regulates cocaine-induced spinogenesis.

Disclosures: S.C. Konefal: None. **J. Clément:** None. **S. Chierzi:** None. **K. Murai:** None. **D. Stellwagen:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant MH104227

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Title: Role of retinoic acid and its receptor on spine plasticity and sensory function in mice

Authors: *M. TJIA[1], E. PARK[2], Y. ZUO[1], L. CHEN[2];
[1]Molecular, Cell and Developmental Biol., Univ. of California Santa Cruz, Santa Cruz, CA;
[2]Departments of Neurosurg. and of Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA

Abstract: Retinoic acid (RA) and its receptors are well-known transcriptional regulators involved in neurogenesis and neural differentiation during embryonic development. Recent findings identify RA and its receptor, RARα, as critical molecules that regulate excitatory and inhibitory synaptic transmission in the context of homeostatic plasticity through modulation of activity-dependent dendritic protein synthesis in the adult brain. Dendritic spines are small protrusions on the dendrites of neurons and serve as the postsynaptic sites of the majority of excitatory synapses in the brain. The role of RA and RARα in homeostatic synaptic plasticity suggest that they might also impact spine plasticity. Combining mouse genetics and *in vivo* two-photon imaging, we followed apical dendrites of layer 5 pyramidal neurons in the barrel cortex of RARα conditional knockout (cKO) mice in which RARα is selectively deleted from the excitatory neurons in the brain. We showed that sensory experience-dependent spine elimination was accelerated in adolescent (1 month old) cKO mice, leading to a decreased spine density in adult (3 Months old) cKO mice. We also show that cKO are less responsive to environmental enrichment, compared to wild-type littermates, suggesting a defective homeostatic regulation. Correlated with the synaptic structural abnormality in the barrel cortex, we also found that cKO mice had impaired whisker-barrel function. These results reveal the important functional role of RA signaling in the developing brain, and identify RARα as an important player in experience-dependent spine pruning.

Disclosures: **M. Tjia:** None. **E. Park:** None. **Y. Zuo:** None. **L. Chen:** None.

Poster

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Title: The inhibition of the EphA4/c-Abl signaling pathway promotes the synaptic insertion of the NMDA-NR2B receptor

Authors: *L. VARGAS ROJAS[1,2], N. LEAL[4,3], P. JIMENEZ[5], F. CARVAJAL[5], W. CERPA[5], A. ALVAREZ[6,3];
[1]Pontificia Univ. Catolica de Chile, Santiago, Chile; [2]Biología Celular y Mol., [3]CENTRO DE ENVEJECIMIENTO Y REGENERACIÓN, CARE, Pontificia Universidad Católica, Chile; [4]Biologia celular y molecular, [5]Pontificia Univ. catolica de chile, Santiago de Chile, Chile; [6]Pontificia Univ. Católica de chile, Santiago de Chile, Chile

Abstract: In the synaptogenesis and synaptic maturation processes, a functional exchange occurs between the subunits of the NMDAR, NR2B and NR2A. The synaptic proteins SAP-102 and PSD-95 participate of the differential transport of the NR2B and NR2A subunits to de synapsis, respectively. In the development stages the NMDAR-NR2B/SAP102 complex are targeted to the synapsis, while the NMDAR-NR2A/PDS-95 complex are inserted in the synapsis during the synaptic maturation process. However, the molecular mechanisms for the development and maturation of the synapsis have not been determined.
The c-Abl kinase translates the signal from various receptors, such as EphA4. We have established that the activation of c-Abl by the EphA4 receptor is fundamental in the dendritic spines loss induced by Aβ oligomers. Additionally, in a physiological context, the activation of the post-synaptic EphA4 receptor by its ligand ephrin-A3, is involved in the retraction and pruning of dendritic spines. EphB2, another member of the ephrin receptors is activated by its specific ligand and induces the increase of NR2B subunit membrane levels in the surface. The activation of the EphB2 receptor leads to Src kinase-dependent phosphorylation of the NR2B in the tyrosine 1472, which is important for the permanence of NMDAR in the surface and its synaptic localization. The activation of EphB2 and EphA4 promote signals which are opposed but necessary to the correct formation of mature synapses.
Our results show that the inhibition of EphA4/c-Abl signaling pathway increase the NMDAR-NR2B/SAP102 complex in the membrane. When the signaling pathway is inhibited using the specific inhibitor KYL for EphA4 and GNF-2 for c-Abl, a significant increase of the NMDA-NR2B and NR2B-phospho-tyr1472 in the synaptic membrane was observed, in primary culture of hippocampal neurons.
Furthermore, in c-Abl knockout mice (cAbl flox/flox Nestin Cre) we observed: i) an increase of NMDA-NR2B and p-NR2B-tyr1472 in the surface of primary cultures and in different days after birth; ii) an increase in the total NMDA currents and iii) additionally we observed that c-Abl knockout mice show high levels of the scaffolding protein SAP102, which is important for the transport of the NR2B subunit.
Interestingly, by inducing the activation of c-Abl with a system inducible by tamoxifen in 293T cells, the phosphorylation in tyrosine 1472 of the NR2B subunit decreases. Summarizing, the activation of the EphA4/c-Abl would be a negative regulator of the synapsis in physiological conditions, because the inhibition of the basal activity of this pathway promotes the insertion in the membrane of the NMDA-NR2B receptors specifically.

Disclosures: L. Vargas Rojas: None. **N. Leal:** None. **P. Jimenez:** None. **F. Carvajal:** None. **W. Cerpa:** None. **A. Alvarez:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Title: A novel DnaJ domain protein regulates activity-dependent synaptic structural modification via integrin activation

Authors: *J. LEE[1], K. T. CHANG[2];
[1]USC, Los Angeles, CA; [2]Zilkha Neurogenetic Institute, Univ. of Southern California, Los Angeles, CA

Abstract: Synapse is a dynamic structure that actively modifies its morphology during development and in response to stimuli. Cell adhesion molecules (CAM) are known to govern such functions by modulating adhesive forces between synaptic terminals. Integrins, trans-synaptic CAMs, have been shown to function in structural remodeling by mediating adhesion between the cell and extracellular matrix (ECM). Here, we demonstrate that a protein named Shriveled, Shv, plays an important role in regulating synaptic growth and activity-dependent synaptic remodeling at the *Drosophila* neuromuscular junction. Depletion of Shv protein results in synaptic overgrowth whereas upregulation of Shv induces enlargement of the synapse instead, a process generally associated with synaptic maturation. We find that Shv genetically interacts with βPS integrin and that Shv is secreted upon intense stimulation to activate integrin signaling. Our data identifies Shv as a novel activator of integrin signaling at the synapse, and further suggests that Shv may restrict synaptic growth and promote synapse maturation. Additional experiments will be conducted to elucidate the mechanism underlying activity-dependent Shv release and integrin activation. Understanding the functional impact of Shv and integrin signaling in synaptic growth may reveal new insights into mechanisms contributing to abnormal structural plasticity that are commonly associated with neurological disorders.

Disclosures: J. Lee: None. **K.T. Chang:** None.

Poster

505. Structural Plasticity II

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Topic: B.08. Synaptic Plasticity

Support: NIH R01 MH099557

Title: Activity-dependent synaptic growth and restructuring in *Drosophila* temperature-sensitive seizure mutant

Authors: *C. L. TORRES FERRERIS[1], A. VASIN[2], M. BYKHOVSKAIA[2];
[1]Anat. and Cell Biol., [2]Neurol., Wayne State University, Sch. of Med., Detroit, MI

Abstract: We investigated how seizure activity affects the neuronal structure at the *Drosophila* larval neuromuscular junction (NMJ). We took advantage of the seizure mutant *(sei)*, which demonstrates neuronal hyperactivity when being exposed to restrictive temperatures. We generated the *Drosophila sei* line with fluorescently tagged neuronal membranes (CD8-GFP). Experiments were performed at dissected preparations with severed axons, as well as at intact larvae. In the latter case, the imaging was performed at anesthetized larvae through the cuticulum. All the NMJs were imaged employing confocal stacks to enable 3D reconstructions of all the synaptic boutons and filopodia. Seizure activity was induced by exposing larvae to restrictive temperatures (50 $^{\circ}$C) for one minute. After a ten minute resting period the preparations were imaged again, and 3D reconstructions of all the neuronal structures were performed. Strikingly, we found a significant restructuring in the stimulated preparations that occurred both in intact and in dissected larvae. The restructuring included the growth of new satellite synaptic boutons and fillopodia and the formation of new boutons along nerve brunches, as well as a retraction of boutons, fillopodia, and even small brunches. These results suggest that seizure activity can produce rapid modifications in the neuronal ultrastructure, including growth and retraction of synaptic boutons. Since such modifications occurred on rather short time scales (ten minutes) and in dissected preparations with severed axons, the underlying mechanisms are likely to be local to the nerve terminals and to involve active transport and trafficking.

Disclosures: C.L. Torres Ferreris: None. **A. Vasin:** None. **M. Bykhovskaia:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH R01 HD67218

Title: The relationship between AMPAR levels and spine dynamics in basal conditions and with motor-skill learning in the fmr1 KO mouse

Authors: *A. SURESH, A. DUNAEVSKY;
Developmental Neurosci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Fragile X syndrome (FXS) is the most common inherited form of intellectual disability. Patients with FXS exhibit a range of neurological deficits including motor skill deficits. Previously, we found that fmr1 knockout mice (KO) have an impairment in motor skill learning in a forelimb reaching task and reduced learning-induced synaptic plasticity in the forelimb area of the primary motor cortex (M1). Specifically, KO mice do not exhibit learning induced increases in dendritic spines as seen in the trained hemisphere of wild type mice and show a delay in synaptic insertion of AMPAR subunit GluA1 in the forelimb region of the primary motor cortex. Here we investigated the relationship between spatiotemporal dynamics of AMPAR and dendritic spine structural plasticity. We performed *in utero* electroporation of AMPAR subunit GluA2 tagged to SEP (GluA2) and a morphological marker td-Tomato to label layer 2/3 neurons in M1 of wild type (WT) littermates and KO mice. We then performed repeated *in vivo* multiphoton imaging of dendritic spines and GluA2 both under basal conditions and following motor skill training in adolescent mice. We find that there is a linear relationship between GluA2 levels and spine volume in both KO and WT controls with a relatively small proportion of spines containing very large amounts of GluA2. In both KO and WT mice the levels of GluA2 were dynamic suggesting that the strength of individual spines varies continuously in adolescent mice. Under basal conditions we find that there is a gradual increase of GluA2 levels in stable spines reaching an average increase of 15% over 10 days in both genotypes. This increase was mainly contributed by low GluA2 containing spines which over time both grew in size and accumulated GluA2. We also observed that in both KO and WT mice spines with large amounts of GluA2 were preferentially stable compared to low GluA2 containing spines. Spine destined for elimination showed decreases in GluA2 hours before elimination. In both KO and WT mice, newly formed persistent spines had increased GluA2 levels 24 hours following formation an additional increases later on. To evaluate learning-induced changes in GluA2, we trained KO and WT mice on a forelimb reaching task. Preliminary results suggest that as in dendrites of layer 5 neurons, motor learning results in an

increase in dendritic spines on layer 2/3 neurons in the trained hemisphere. Moreover, an increase in synaptic GluA2 was observed in wild type mice but not in the KO mice. Hence our results suggest that under basal conditions the relationship between AMPAR and dendritic spines is not altered in the fmr1 KO mice but may be affected under activity dependent mechanisms such as learning.

Disclosures: **A. Suresh:** None. **A. Dunaevsky:** None.

Poster

505. Structural Plasticity II

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Topic: B.08. Synaptic Plasticity

Title: Subchronic low-dose ketamine stimulates formation of dendritic spines in somatosensory cortex of awake behaving mice

Authors: ***L. KHIROUG**[1], E. PRYAZHNIKOV[3], P. CASAROTTO[2], J. KOLIKOVA[3], P. MARSHALL[3], D. TOPTUNOV[3], P. HOTULAINEN[4], V. VOIKAR[2], R. TERRY-LORENZO[5], S. ENGEL[5], E. CASTREN[2];
[2]Neurosci. Ctr., [1]Univ. of Helsinki, Helsinki, Finland; [3]Neurotar Ltd, Helsinki, Finland; [4]Minerva Fndn. Inst. for Med. Res., Helsinki, Finland; [5]Sunovion Pharmaceuticals Inc, Marlborough, MA

Abstract: Our primary finding is that subchronic 5-day once-daily treatment with ketamine stimulates spine formation in mouse primary somatosensory cortex, reversing the net loss of spines (synaptic pruning) that is observed in control animals at this age (2-3 m.o.). Ketamine has attracted significant attention as a drug of abuse and, more recently, as a rapidly acting antidepressant. However, the structural synaptic plasticity mechanisms induced by repeated ketamine administration remain unknown. We studied effects of subchronic ketamine injections on spine formation and elimination rates in awake, behaving mice. Young adult female mice expressing YFP in cortical layer 5 pyramidal neurons were head-fixed in the Mobile HomeCage device enabling microscopic imaging in the cortex of awake behaving mice. Ketamine (10mg/kg) or vehicle (PBS) was injected intraperitoneally and spines were imaged at 0h, 6h, 24h, 72h and 120h, with ketamine/vehicle treatment at 1h, 25h, 49h, 73h and 97h. Apical dendrites of layer 5 neurons in primary somatosensory cortex were imaged in layers 1 and 2. Between 150 and 300 spines per animal were tracked individually over time. Ketamine significantly increased the spine formation rate at 72h ($p<0.05$, $n=6$ mice for ketamine group, $n=5$ mice for control group). We also observed a strong trend towards the decrease in the rate of spine elimination, although this effect of ketamine did not reach the significance level. As a

whole, the control group displayed a net loss of spines, and this loss was reversed by ketamine. Effects of ketamine on spine formation in somatosensory cortex correlated with ketamine-induced increase in phospho-Actin and PSD-95 protein level ($p<0.05$ at 72h), consistent with formation of functional synapses. Interestingly, the protein level of NR2B subunit of NMDA receptor was not affected by subchronic 10mg/kg ketamine treatment. However, the ratio between mobile and stable spine fractions was unaffected by ketamine treatment. Behavioral tests revealed that ketamine injections induced a sustained significant improvement in the nest score without affecting the overall activity of the animals.

Disclosures: **L. Khiroug:** None. **E. Pryazhnikov:** None. **P. Casarotto:** None. **J. Kolikova:** None. **P. Marshall:** None. **D. Toptunov:** None. **P. Hotulainen:** None. **V. Voikar:** None. **R. Terry-Lorenzo:** None. **S. Engel:** None. **E. Castren:** None.

Poster

505. Structural Plasticity II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 505.23/M12

Topic: B.08. Synaptic Plasticity

Support: NIMH

NINDS

Title: Environmental enrichment confers resistance to stress-induced abnormal dendritic spine dynamics and cognitive defects in the mouse cerebral cortex

Authors: ***C.-C. CHEN**, Y. ZUO;
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Abstract: It is widely accepted that significant experiences can rapidly cause long-lasting alterations of anatomical, physiological, and behavioral features in the brain. As one of the most prevalent experiences in modern society, stress can have a profound and enduring impact on brain functioning. These functional defects are primarily mediated through the dysregulation of synapses, the communication sites between neurons. Much is known about the deleterious effects of stress on the affective systems mediated through subcortical structures, but less has been explored in the cortical regions, which are the seats for perception, memory, and cognition. Furthermore, it is unclear how positive experiences may counteract or negate the effects of stress. To address these questions, we investigated how various experiences, stressful and/or enriching, can alter cortical functioning and its associated synapse dynamics. Using *in vivo* two-

photon transcranial microscopy, we longitudinally followed the dynamics of postsynaptic dendritic spines of apical dendrites in layer V pyramidal cortical neurons. We found that animals reared in an enriched environment are more resistant to stressful experiences than animals reared in standard cages, measured by a cortex-specific cognitive task. Similarly, enriched environment also counteracts the negative effects of stress on dendritic spines. Understanding how various experiences cooperate or negate one another, provides a framework on how similar or opposing events in life can differentially leave lasting footprints in the neurocircuit, thus impacting cognitive processes through the altered functioning of synaptic structures.

Disclosures: C. Chen: None. **Y. Zuo:** None.

Poster

505. Structural Plasticity II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 505.24/M13

Topic: B.08. Synaptic Plasticity

Support: NIH/NIMH grant R01-MH100561

Title: Synaptic pruning in layer 5 of the medial prefrontal cortex of female mice requires $GABA_A$ receptors

Authors: *M. R. EVRARD, S. S. SMITH;
Physiol. and Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY

Abstract: The onset of certain neurodevelopmental disorders, such as schizophrenia, overlaps with the maturation of the prefrontal cortex (PFC), suggesting that it is implicated in their etiology. Unlike other areas of the brain, the PFC is not fully developed until early adulthood. It is well established that before puberty the number of synaptic spines located on the dendrites of cortical pyramidal cells increases dramatically. These synaptic connections that develop before puberty are pruned during adolescence (Huttenlocher, 1979; Koss, et al., 2014); this process, known as synaptic pruning, is thought to be important for normal cognition because dysregulation of this process appears to result in neurodevelopmental disorders. However, the initial mechanisms which trigger synaptic pruning remain largely unknown. It is known that the spines of cortical pyramidal cells receive GABAergic input. We first identified layer-specific pruning in the mPFC using the Golgi-Cox staining method in female mice at puberty (PND 35, confirmed by vaginal opening) and post-pubertally (PND 56). Spine counts were obtained using a Nikon Eclipse Ci-L microscope and manually counted on basilar dendrites. Spine density in layer 5 of the mPFC decreased by 62% from puberty (5.9 spines/10 μm) to post-puberty (3.7

spines/10 μm; $P < 0.0001$) - with the greatest decline occurring in the medial (Puberty, 7.4 spines/10 μm; Post-puberty, 3.6 spines/10 μm, $P < 0.0005$) and distal (Puberty, 7.5 spines/10 μm; Post-puberty, 4.9 spines/10 μm, $P < 0.0005$) regions. To test the hypothesis that synaptic pruning is $GABA_A$ receptor (GABAAR) mediated we injected female mice with picrotoxin (3mg/kg, i.p.), a GABAAR antagonist, once a day for the first 10 days following the onset of puberty (P36-P46); mice were then sacrificed at P56. Spine density in mice injected with picrotoxin had a significantly higher synaptic density in each region (proximal, 5.4 spines/10 μM [$P < 0.0001$]; medial, 8.7 spines/10μm [$P < 0.0001$]; distal, 11.3 spines/10μm [$P < 0.0001$]) and overall (8.5 spines/10μm, $P < 0.0001$) than untreated post-pubertal mice. These data suggest that synaptic pruning occurs in layer 5 of the prefrontal cortex in female mice and is mediated by GABAARs, which prior to this study, has not been shown. Future studies seek to identify specific GABAARs that mediate synaptic pruning.

Disclosures: **M.R. Evrard:** None. **S.S. Smith:** None.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

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Program#/Poster#: 506.01/M14

Topic: B.10. Network Interactions

Support: NARSAD Independent Investigator Award

Swiss National Science Foundation P300PA_164693

Title: Pilot clinical trial of transcranial alternating current stimulation (tACS) for the treatment of major depressive disorder

Authors: ***C. E. LUGO**, J. MELLIN, M. ALEXANDER, S. ALAGAPAN, C. LUSTENBERGER, D. RUBINOW, F. FROHLICH;
psychiatry, UNC, Raleigh, NC

Abstract: Major depressive disorder (MDD) is a network disorder characterized by impaired organization of electric activity patterns in the brain. Given the known alterations in alpha oscillations (8-12 Hz) [1] in MDD, we hypothesized that transcranial alternating current stimulation (tACS) can alter alpha oscillations and improve clinical symptoms in people with MDD. To test this hypothesis, we are conducting a double-blind, sham-controlled pilot clinical trial to establish feasibility and to collect initial efficacy data for the use of tACS in MDD patients. The study employs a parallel design with three arms; participants receive daily 40

minutes stimulation for 5 consecutive days (40Hz gamma tACS, 10 Hz alpha tACS or active sham). The stimulation frequencies were chosen due to the emerging evidence that tACS in these frequencies modulate alpha oscillations in healthy controls. Changes in the Montgomery Asberg Depression Rating Scale (MADRS) scores from baseline to the 4 week follow-up visit are the primary outcome. Changes in resting-state alpha oscillation power from high-density EEG are the secondary outcome. In addition, a working memory task (n-back task) is administered during each EEG recording. Data from the Beck Depression Inventory (BDI), Clinical Global Impression (CGI), Hamilton Depression Rating Scale (HDRS), Montreal Cognitive Assessment (MOCA), and Young Mania Rating Scale (YMRS) are also collected. This study is currently enrolling patients (8 patients enrolled, target: 21 by Nov 2016). Here, we report data from the first 6 patients who have completed the stimulation week (all female, mean age 34.7 years). At baseline, the mean MADRS was 23.2 (std: 6.9) and the mean HDRS was 14.2 (std: 5.1). At the completion of the stimulation week, the mean MADRS was 16.3 (std: 5.1) and the mean HDRS was 9.8 (std: 3.9). At baseline, the mean for the MOCA was 27.2 (std: 1.9). All patients had a score of zero on the YMRS both at baseline and at completion of the stimulation week. Self-reported depression symptoms assessed by the BDI decreased from 25.2 (std: 5.4) before stimulation to 16.5 (std: 9.3) at the end of the stimulation week. Due to the small sample size and the fact that the data are blinded, no conclusion about efficacy can be drawn at this point. However, our early results demonstrate that the treatment is safe and well tolerated and that overall depression symptoms improved in the patients enrolled in this study. Leuchter et al. (2013). Frontiers in Human Neuroscience, 7.

Disclosures: **C.E. Lugo:** None. **J. Mellin:** None. **M. Alexander:** None. **S. Alagapan:** None. **C. Lustenberger:** None. **D. Rubinow:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro, LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 506.02/M15

Topic: B.10. Network Interactions

Support: NIH Grant R01MH101547

NIH Grant 2R01EB016407

Swiss National Science Foundation P300PA_164693

UNC Psychiatry

UNC School of Medicine

Title: Auditory steady state responses during wakefulness and nrem sleep

Authors: *C. LUSTENBERGER[1], Y. A. PATEL[2], J. M. PAGE[1], B. PRICE[1], S. ALAGAPAN[1], M. R. BOYLE[1], F. FROHLICH[1];
[1]UNC at Chapel Hill, Chapel Hill, NC; [2]Georgia Inst. of Technol., Atlanta, GA

Abstract: Rhythmic sensory stimulations are able to drive brain oscillations by increasing phase-locking to the temporal structure of the stimulation and by increasing power of specific frequency bands [1]. Since distinct intrinsic oscillations such as alpha oscillations (8-12 Hz) and sleep spindles (11-16 Hz) are characteristic of different vigilance states [2], it remains to be investigated whether rhythmic stimuli elicit different brain responses during these states. Here, we compared the effects of auditory rhythmic stimuli on EEG brain activity during wake and sleep with a specific focus on the spindle frequency range, and delineated the topographical distribution of these modulations. We included 8 healthy, male participants in our study (18-29 years). During the wakefulness session (30 min), amplitude modulated (AM) white noise stimuli at different frequencies were applied for 1 s with inter-trial intervals of 2.5-3.5 s. The same auditory paradigm was repeated during a 60 minute nap. 128-channel high-density EEG was continuously recorded during the nap and wakefulness sessions. Event-related spectral power (ERSP) and inter-trial phase coherence (ITPC) analyses were performed for artifact-free wakefulness and NREM events in the spindle frequency range (11-16 Hz). We focussed our analysis on 14 Hz AM stimuli. ITPC at 14 Hz was more pronounced during wakefulness than sleep for a broad fronto-central cluster of electrodes (maximal ITPC difference +11%, $p<0.05$, paired t-test). However, ERSP increase in the slow spindle frequency range (11-13 Hz) relative to a pre-event baseline was significantly stronger for events applied during NREM sleep and nearly absent during wakefulness. The topography of this slow spindle activity increase was most distinct over fronto-central regions (maximal ERSP difference + 16%, $p<0.05$), which is characteristic for slow spindles. A similar picture emerges for the fast spindle frequencies (14-16 Hz). ERSP was again significantly increased during NREM sleep compared to wakefulness (maximal ERSP difference +25%, $p<0.05$) but with a distinct centro-parietal topography representative for fast sleep spindles. In conclusion, brain responses to temporally patterned auditory stimuli were different for wakefulness and NREM sleep and therefore specific to vigilance states. Strikingly, spindle-like AM stimuli increased spindle activity during NREM sleep but not during wakefulness. Thus, auditory stimulation during sleep might represent a powerful tool to boost sleep spindles and test their functional role in memory and cognition.
[1] Thut G., et al. (2011). Front Psychol, 2. [2] Steriade M., et al. (2003). Neuron, 37.

Disclosures: **C. Lustenberger:** None. **Y.A. Patel:** None. **J.M. Page:** None. **B. Price:** None. **S. Alagapan:** None. **M.R. Boyle:** None. **F. Frohlich:** B. Contracted Research/Research Grant

(principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro, LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 506.03/M16

Topic: B.10. Network Interactions

Support: NIH grant R21MH105557

Title: Entrainment of thalamocortical networks by tACS is modulated by intra-area coupling

Authors: *C. HENRIQUEZ[1], G. LI[2], F. FROHLICH[2];
[1]Biomed. Engin., Duke Univ., Durham, NC; [2]Univ. of North Carolina, Chapel Hill, NC

Abstract: Thalamocortical dysrhythmia has been proposed as a mechanism underlying neurological and psychiatric conditions. These conditions have been associated with persistent low-frequency activity that limits normal cognitive function, and may be a result of asymmetric synaptic coupling between the thalamus and cortical layers. We hypothesize modulation of the thalamocortical circuit using transcranial alternating current stimulation (tACS) depends on the degree of the asymmetry, since the current will mostly affect the closer cortical cells.
To test this hypothesis, we developed a biophysical thalamocortical model consisting of four, two-dimensional, layer networks consisting of a cortical region (CX) comprised of 225 pyramidal (PY) cells, 100 cortical interneurons (IN), and a thalamic region (Th) comprised of 100 reticular cells (RE) and 49 high threshold bursting thalamocortical (HTC) cells. Between CX and TH, HTC cells project with glutamatergic synapses to the both the PY and IN cells (HTC-CX) while the PY cells project back with glutamatergic synapses (PY-TH) to both the RE and HTC cells. A simulated LFP was computed by low-pass filtering the synaptic currents in the PY layer and used to compute the power spectral density (PSD). A dysrhythmic network was created by enhancing the HTC-CX and RE to HTC coupling and reducing the PY-TH coupling. In both normal and dysrhythmic alpha states, the TH and CX networks oscillated coherently at 10 Hz, but the oscillatory power was much stronger in the dysrhythmic condition. In the normal gamma state, the CX synchronizes the TH network at 32Hz, while under the dysrhythmic gamma state, the CX gamma oscillation failed to synchronize the TH network, which oscillated at 14Hz. As a

result, a strong 14 Hz peak appeared in the CX PSD along with the dominant 32 Hz peak. A sinusoidal current, 5Hz above the endogenous CX frequency, was applied to only the PY cells and the minimum current needed for entrainment was determined. Results showed that the dysrhythmic gamma state needed 15% lower current to entrain than the normal gamma state while the dysrhythmic alpha state needed 350% higher current to entrain than the normal alpha state. The findings suggest that in a dysrhythmic alpha state in which TH more strongly drives CX rhythm, it is more difficult to perturb the endogenous rhythm with tACS that targets the cortical cells. In contrast, for the dysrhythmic gamma state where the dominant CX rhythm is not synchronized with the TH rhythm, entrainment is easier. The development of models that are capable of generating a variety of dysrhythmic conditions may be valuable in designing more optimal stimulation therapies.

Disclosures: C. Henriquez: None. **G. Li:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro, LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: B.10. Network Interactions

Support: UNC Psychiatry, UNC School of Medicine, a donation by Dean and Brenda Proctor, the Human Frontier Science Program, and by the NIMH Award R01MH101547

Title: Task dependent modulation of neural activity in the ferret lateral posterior pulvinar complex during preparatory attention

Authors: *C. YU[1], I. M. STITT[2], Y. LI[2], Z. ZHOU[2], K. K. SELLERS[2], F. FROHLICH[2];
[1]Dept. of Psychiatry, [2]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: The pulvinar plays a key role in organizing cortico-cortical interactions during attention-demanding tasks in primates[1] .Little is known about the circuit dynamics within the pulvinar and its role in attentional behavior. Here, we performed multichannel electrophysiological recordings in the lateral posterior-pulvinar complex (LP/pulvinar) in the

ferret (*Mustela putorius furo*). We recorded single unit activity and local field potential during the performance of the touch screen version of 5-choice serial reaction time task (5-CSRTT). We found that freely moving, trained ferrets were able to consistently perform the task. Attention responsive neurons (n=130) clearly responded while the animal maintained attention during the delay period when no stimuli were present and exhibited a monotonically increasing firing rate during that period. Neurons non-responsive to attention (n=129) signaled in response to the stimulus, but not during the delay period. Spike-field coherence of the responsive neurons significantly increased during attention period until screen touch, predominantly in the theta frequency band (~5Hz). Furthermore, we found that theta power and theta-gamma phase amplitude coupling was substantially elevated throughout the delay period. Interestingly, the strength of the power in the theta frequency band was positively correlated with reaction time from stimulus onset to screen touch. Together, our results reveal task-dependent LP/Pulvinar theta oscillations in the freely moving ferret while performing an attention demanding task. Our findings further suggest that the theta oscillation may play an important role in coordinating cortical activity in the ferret. Reference: 1.Saalmann, Y.B., et al., The pulvinar regulates information transmission between cortical areas based on attention demands. Science, 2012. 337(6095): p. 753-6.

Disclosures: **C. Yu:** None. **I.M. Stitt:** None. **Y. Li:** None. **Z. Zhou:** None. **K.K. Sellers:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pulvinar Neuro, LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 506.05/M18

Topic: B.10. Network Interactions

Support: NIH Grant 1R21MH105574-01

Title: Targeting auditory hallucinations with tdcs and tacs

Authors: *J. M. MELLIN, C. E. LUGO, M. L. ALEXANDER, S. ALAGAPAN, C. LUSTENBERGER, J. H. GILMORE, L. F. JARSKOG, F. FROHLICH;
Psychiatry, Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: Approximately 30% of patients with schizophrenia experience auditory hallucinations that are refractory to antipsychotic medications and cause a significant decrease in the quality of life [1]. Thus far, attempts for using transcranial current stimulation as a novel treatment have been limited to transcranial direct current stimulation (tDCS) [2]. A previous study conducted by Brunelin et al. [2] found a significant reduction in auditory hallucinations in people with schizophrenia after 5 days of twice daily tDCS that targeted both the left dorsolateral prefrontal cortex (dl-PFC) and the left temporo-parietal cortex. In contrast, a pilot study conducted in our laboratory found no difference in auditory hallucinations between once daily tDCS and sham as measured by the Auditory Hallucination Rating Scale (AHRS) [3]. Here, we evaluated the efficacy of transcranial alternating current stimulation (tACS) that we hypothesized would enhance synchronization between the frontal and temporo-parietal areas of the left hemisphere. In addition to behavioral assessments, EEG data were collected to determine if and how stimulation engaged the network-level stimulation targets. The study had a parallel group design with three arms (sham, tDCS, and tACS). Participants received twice daily, 20 minute sessions of active sham, 10 Hz 2mA peak-to-peak tACS, or 2 mA tDCS over the course of 5 consecutive days.Both high-density EEG and behavioral measures were measured to determine the efficacy of tDCS and tACS for the treatment of medication-refractory auditory hallucinations in patients with schizophrenia. The AHRS and EEG recordings were the primary outcomes for this study. The AHRS was administered at the baseline visit, the first and last day of stimulation, and at the one week and one month follow-up visits. EEG data was collected at the first and last day of stimulation, and at the one week and one month follow-up. Data from the Positive and Negative Syndrome Scale (PANSS), Brief Assessment of Cognition in Schizophrenia (BACS), Abnormal Involuntary Movements Scale (AIMS), Simpson Angus Scale (SAS), and Clinical Global Impression (CGI) were collected at the first and last day of stimulation, and at the one month follow-up. This study is currently enrolling participants, and the data remains blinded. Unblinded results will be presented at the meeting. 1. Shergill et al. (1998). Schizophrenia Research, 32(3). 2. Brunelin et al. (2012). The American Journal Of Psychiatry, 169(7). 3. Frohlich et al. (2016). European Psychiatry, 33.

Disclosures: **J.M. Mellin:** None. **C.E. Lugo:** None. **M.L. Alexander:** None. **S. Alagapan:** None. **C. Lustenberger:** None. **J.H. Gilmore:** None. **L.F. Jarskog:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro, LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 506.06/N1

Topic: B.10. Network Interactions

Title: Fluctuations in neuromodulatory tone shape the dynamics of thalamocortical functional interaction

Authors: *I. M. STITT, Z. ZHOU, F. FROHLICH;
Dept. of Psychiatry, UNC, Chapel Hill, NC

Abstract: The brain continually transitions between states of varying arousal. These ongoing shifts in brain state are partially caused by changes in neuromodulatory drive to cortical and subcortical structures[1]. Converging evidence supports that neural activity in the locus coeruleus - the main source of noradrenergic input to the rest of the brain[2] - is correlated with fluctuations in pupil diameter[3]. Previous work linking fluctuations in pupil diameter and neural activity have primarily found that noradrenergic tone shapes the intrinsic properties of individual neurons[1]. However, state dependent shifts in behavior emerge from large-scale patterns of neural activity coordinated across many interconnected brain regions. Here, we hypothesize that spontaneous fluctuations in noradrenergic tone shape the dynamics of thalamo-cortical functional interaction. We recorded neural activity simultaneously from the lateral posterior/pulvinar nucleus in the thalamus and posterior parietal cortex of awake head-fixed ferrets. The noradrenergic state of the animal was continuously monitored by tracking of the pupil diameter under constant luminance. We found that local field potential (LFP) power and neuronal firing rate in both cortex and thalamus were significantly modulated with changes in pupil diameter ($p<0.01$). Thalamo-cortical LFP phase synchronization was observed at theta (4Hz) and alpha frequencies (14Hz) for low pupil dilation. However, as pupil diameter increased, alpha phase synchronization significantly decreased, while theta phase synchronization significantly increased ($p<0.01$). Further, LFP amplitude envelope correlation analysis revealed a strong antagonism between thalamic theta and cortical alpha rhythms. In contrast to the LFP, spike-spike and spike-LFP functional connectivity analyses predominantly revealed interactions in the alpha band. The strength of all spiking based functional connectivity analyses in the alpha band significantly decreased with pupil dilation ($p<0.01$). Collectively, these results show that neuromodulatory factors play a critical role in shaping the dynamics of thalamo-cortical functional interaction. Indeed, by extension, our findings give rise to the notion that complex patterns of brain dynamics may be readily inferred via the easily measurable external variable of pupil dilation.
1 McGinley, M. J. *et al*. *Neuron* **87**, 1143-1161 (2015).
2 Schwarz, L. A. *et al. Nature* **524**, 88-92, (2015).
3 Joshi, S. *et al*. *Neuron* **89**, 221-234, (2016).

Disclosures: **I.M. Stitt:** None. **Z. Zhou:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro, LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 506.07/N2

Topic: B.10. Network Interactions

Support: NIH R01MH101547

Title: Rhythmic interactions between the higher-order visual thalamus and the posterior parietal cortex influence saccadic sampling

Authors: ***Z. C. ZHOU**, I. M. STITT, F. FROHLICH;
Psychiatry, Univ. of North Carolina - Chapel Hill, Chapel Hill, NC

Abstract: Saccades, a form of visual sampling, serve to evaluate the dynamically changing external environment. Cortical neural activity is implicated in the voluntary control of gaze, whereas subcortical regions are important for the execution of saccades [1,2]. Neural activity and saccade-related behavior may be linked by common periodic rhythms [3,4]. However, little is known about how saccades are modulated by the interaction between subcortical and cortical regions, specifically in the context of rhythmic activity. To investigate this, we simultaneously recorded multi-unit and local field potential (LFP) activity from the lateral posterior/pulvinar nucleus (LP/pulv) and the posterior parietal cortex (PPC) in head-fixed ferrets. During these recordings, two experimenters engaged in verbal discourse in front of the animal in order to elicit visual sampling from the ferret in either lights-on or lights-off conditions. Coordinates of the pupil center were recorded using an eye-tracking system (ISCAN,Woburn,MA). We found peaks in the inter-saccade interval at around 250 ms (4 Hz, lights-on) and 160 ms (6.3 Hz, lights-off), and a strong correlation between saccade velocity and magnitude (lights-on: $r=0.87$, $t=134$, $p<0.0001$; lights-off: $r=0.88$, $t=151$, $p<0.0001$), similar to measurements recorded from humans and non-human primates. In cortex, we observed a transient increase in high frequency (20-100Hz) LFP power about 100ms in duration, and a decrease in low frequency (2-18Hz) LFP power about 1s in duration after the saccade. In LP/pulv, we found an enhancement of power in

the theta band throughout the duration of the saccade (4.4 Hz power, baseline vs saccade, t(26)=-5.399, p<0.0001). Importantly, thalamocortical phase synchronization in the theta band (4.4 Hz) increased prior to saccades and remained elevated for up to 1s, indicating the co-occurrence of dynamic LP/pulv and PPC functional interaction and saccadic sampling. As a whole, our study reveals that thalamo-cortical rhythms influence the integration of saccadic sampling and processing of visual input; importantly, these results may inform the study of the mechanisms underlying visual attention-deficit disorders.

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2. Van Der Werf, J., Jensen, O., Fries, P. & Medendorp, W. P. *J Neurosci* **28**, 8397-8405, (2008).
3. Bosman, C. A., Womelsdorf, T., Desimone, R. & Fries, P. *J Neurosci* **29**, 9471-9480, (2009).
4. Otero-Millan, J., Troncoso, X. G., Macknik, S. L., Serrano-Pedraza, I. & Martinez-Conde, S. *J Vision* **8**, 21 21-18, (2008).

Disclosures: Z.C. Zhou: None. **I.M. Stitt:** None. **F. Frohlich:** None.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

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Topic: B.10. Network Interactions

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Title: Enhancement of working memory performance by intracranial periodic pulse stimulation in humans

Authors: *S. ALAGAPAN[1], E. HADAR[2], H. SHIN[3], F. FROHLICH[4];
[1]Univ. of North Carolina At Chapel Hill Sch., Carrboro, NC; [2]Neurosurg., [3]Neurol., [4]Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Working memory (WM), an integral component of higher-order cognition, is impaired in many neurological and psychiatric disorders. The neural correlates of WM performance often include low-frequency cortical oscillations (4 - 12 Hz) which tend to be aberrant in disorders with impaired WM. Thus these oscillations might serve as targets for treating WM deficits [1]. The combination of electrocorticography (ECoG) and direct cortical stimulation (DCS) offers a platform to target specific areas and specific activity patterns in humans. In this study, we

present results from a participant with subdural electrodes who underwent periodic pulse stimulation of frontal and temporal cortices when performing a Sternberg verbal working memory task.
The participant, a 23 year old right handed female, was implanted with subdural and depth electrodes covering bilateral frontal, temporal, parietal cortices and hippocampi for epilepsy surgery planning. The participant performed a Sternberg verbal working memory task with randomized list lengths of 3, 4 and 5. Concurrently, electrical stimulation was applied to predetermined pairs of electrodes synchronized with the task. Stimulation consisted of 5 second long trains of 2 mA biphasic electric pulses 200 μS in duration at 10 Hz (1 pulse every 100 ms). Also, sham trials with no electrical stimulation were randomly interleaved with the patient blinded to the condition. We found that stimulation did not alter accuracy (Stim: 95.94 ± 2.17 % vs Sham: 98.48 ± 1.52 % Sham). However, stimulation resulted in a decrease in reaction times for trials in which list lengths were 4 (Stim: 988 ± 56 ms vs Sham: 1119 ± 29 ms; $p = 0.39$, two sample t-test) and 5 (Stim: 899 ± 49 ms vs Sham: 1140 ± 22 ms; $p = 0.01$). In contrast, the reaction times for lists of length 3 did not show any difference (Stim: 826 ± 58 ms vs Sham: 824 ± 9 ms; $p = 0.98$). Concurrently, the task-related signal power change in low frequency band (4 - 12 Hz) during stimulation measured by modulation index was higher compared to sham in the trials where list length was 4 (Stim: -0.003 ± 0.005 vs Sham: -0.015 ± 0.006; $p = 0.13$, two sample t-test) and 5 (Stim: 0.071 ± 0.005 vs Sham: 0.007 ± 0.006; $p < 0.001$) and there was no difference between the signal power change in trials where list length was 3 (Stim: - 0.027 ± 0.006 vs Sham: -0.026 ± 0.005; $p = 0.90$). The results suggest stimulation induced changes in oscillation power may be linked to the observed changes in reaction times. Thus, our data demonstrate the possible role oscillation power may play in cognition and the feasibility of periodic stimulation to modulate oscillation power and in turn cognitive performance.
1. Luber, B., et al., Brain Research, 2007. 1128(1)

Disclosures: **S. Alagapan:** None. **E. Hadar:** None. **H. Shin:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

506. Oscillations and Synchrony: Other I

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Topic: B.10. Network Interactions

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Human Frontier Science Program

UNC Department of Psychiatry

UNC School of Medicine

Donation by Dean and Brenda Proctor

Title: Oscillatory interaction dynamics in the frontoparietal attention network during sustained attention in the ferret

Authors: *K. K. SELLERS, C. YU, Z. C. ZHOU, I. M. STITT, Y. LI, S. RADTKE-SCHULLER, S. ALAGAPAN, F. FROHLICH;
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Abstract: Sustained attention requires the organization of activity across multiple cortical areas in the frontoparietal network [1]. Two brain regions critical for sustained attention are prefrontal cortex (PFC) and posterior parietal cortex (PPC). Previous work has demonstrated the coordination of these brain areas during attention at the level of local field potential (LFP) synchronization [2, 3]. However, the underlying mechanisms of these interaction dynamics in terms of organization of single unit activity (SUA) have remained poorly understood. To fill this gap, we asked how LFP and SUA organize local and long-range interactions in PFC and PPC. We hypothesized that local organization of SUA is mediated by high-frequency oscillatory activity and that long-range organization relies on low-frequency oscillations.
We simultaneously recorded SUA and LFP activity in PFC and PPC of three ferrets (*Mustela putorius furo*) during the five-choice serial reaction time task, a sustained attention task [4]. In a separate set of experiments, we established structural connectivity between these areas using anterograde tracer rAAV5-CamKII-GFP and retrograde tracer Alexa 488-CTB. We found modulation of SUA (87% of PFC units, 85% of PPC units) during the task. In PPC, there were local spectral peaks in the LFP at 5Hz and in the gamma band, with a modest power increase in power during the sustained attention period (PPC 5Hz theta, $t(41) = -6.62$, $p < 0.001$; PPC gamma, $t(41) = -10.30$, $p < 0.001$; PPC 80-120Hz high gamma, $t(41) = -6.73$, $p < 0.001$). Long-range functional connectivity between PFC and PPC was mediated by low-frequency oscillations at 5Hz. In agreement with our hypothesis, we found that SUA in PFC was organized by 5Hz theta oscillations in PPC (30.5% of units exhibited significant spike-LFP phase locking) and locally by both theta and high-gamma oscillations (25.5% and 57.5-72.9% of units, respectively). SUA in PPC was only guided locally by high-gamma oscillatory activity (58.0-70.0% of units). To our knowledge, this is the first multisite recording in PFC and PPC during a sustained attention task in the moving animal. Most strikingly, activity in the theta frequency band emerged as the fundamental rhythm that organized both local and long-range interactions in

these areas. These results support a crucial role of theta oscillations in organizing large-scale functional networks during behavior.

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2. Buschman, T.J. and E.K. Miller. Science, 2007. 315(5820): p. 1860-2.
3. Phillips, J.M., et al. Cereb Cortex, 2014. 24(8): p. 1996-2008.
4. Bari, A., J.W. Dalley, and T.W. Robbins. Nat Protoc, 2008. 3(5): p. 759-67.

Disclosures: K.K. Sellers: None. **C. Yu:** None. **Z.C. Zhou:** None. **I.M. Stitt:** None. **Y. Li:** None. **S. Radtke-Schuller:** None. **S. Alagapan:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro, LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

506. Oscillations and Synchrony: Other I

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Topic: B.10. Network Interactions

Support: NIH Grant R01MH101547

NIH Grant R21MH105557

Title: Targeting alpha oscillations with amplitude-modulated transcranial alternating current stimulation (AM-tACS)

Authors: *E. NEGAHBANI[1], F. KASTEN[2], C. HERRMANN[3,4], F. FROHLICH[1];
[1]Dept. of Psychiatry, Univ. of North Carolina, Chapel Hill, NC; [2]Dept. of Psychology, [3]Exptl. Psychology Lab, Ctr. for excellence 'Hearing4all', European Med. Sch., [4]Res. Ctr. Neurosensory Sci., Carl von Ossietzky Univ., Oldenburg, Germany

Abstract: Endogenous brain oscillations can be entrained by externally applied weak electrical fields through transcranial alternating current electrical stimulation (tACS). Many studies have focused on comparison of neuronal activity between pre and post-stimulation periods, but the underlying mechanisms of tACS may be best understood by analysis of neural data during tACS. However, the pronounced stimulation artifact makes the study of the network dynamics during

tACS challenging. Here we use computer simulations to investigate a recently proposed solution to avoid such artifact issues. The nonlinear properties of the neuronal cell membrane make neurons responsive to envelope frequency of a modulated sensory input as reported in auditory and visual cortices [1]. Accordingly it is suggested that neurons might also be susceptible to modulating frequency of an amplitude-modulated tACS (AM-tACS) input [2]. AM-tACS enables a separation of the frequency of stimulation (FOS) from the frequency of interest (FOI) by selecting a modulating sinusoidal at the FOI and a high-frequency carrier [2]. We evaluated this approach with computer simulations of a cortical network and AM-tACS. The cortical model was set to exhibit an oscillation with an endogenous frequency of f_{end}~7.8 Hz. We first examined classical tACS with different amplitudes and frequencies resulting in entrainment delineated in the parameter space as an Arnold tongue centered around f_{end}. Next we applied AM-tACS with fixed modulating frequency of f_m=f_{end} and different stimulation amplitudes and carrier frequencies f_c. The result indicates filtering of high-frequency stimuli in agreement with the low-pass filtering by the neuronal cell membrane. We then applied AM-tACS with fixed fc=55 Hz and different f_m and stimulation amplitudes. The resulting Arnold tongue indicates clear entrainment around f_{end} induced by the low-frequency envelope of AM-tACS. Together, these results show that the envelope of AM-tACS can entrain neural oscillators at their endogenous frequency however the stimulation amplitudes need to be increased to compensate for the low-pass filtering of the neuronal cell membrane.

1. Middleton, J.W., et al. Proc Natl Acad Sci U S A, 2006. 103(39): p.14596-14601.
2. Witkowski, M., et al. NeuroImage 2015.

Disclosures: **E. Negahbani:** None. **F. Kasten:** None. **C. Herrmann:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro, LLC. Other; Strata Solar.

Poster

506. Oscillations and Synchrony: Other I

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Topic: B.10. Network Interactions

Support: NIH Grant R01MH101547

Swiss National Science Foundation P300PA_164693

UNC Psychiatry

UNC School of Medicine

Title: Evaluating transcranial alternating current stimulation (tACS) for enhancing creativity

Authors: *B. PRICE, C. LUSTENBERGER, S. ALAGAPAN, F. FROHLICH;
Psychiatry, Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: Because creativity is an abstract idea indicative of higher order thought, it can be difficult to evaluate scientifically. However, as the workings of brain electrophysiology are studied, understanding the biology underlying creativity has become possible. We have previously shown that targeting bilateral frontal alpha activity using frequency-specific transcranial alternating current stimulation (tACS) resulted in enhanced creativity when compared with a 40 Hz sham condition [1]. EEG studies suggest that there are differences in the way that alpha oscillations mediate creativity in the left and right hemispheres, with a greater amount of alpha synchronization in the right hemisphere than the left hemisphere [2]. To further investigate the role of hemispheric alpha in creativity, we applied unilateral tACS to target the middle frontal gyrus.

A total of twenty healthy, male and female subjects over the age of eighteen were stimulated with tACS while taking the figural TTCT (Torrance Tests for Creative Thinking). Participants received 2 mA peak-to-peak alpha tACS (10 Hz) at either left frontal or right frontal locations, with a common electrode (Cz) over the apex. During the first round of stimulation, participants took one test form (A or B) of the TTCT. A thirty-minute resting period followed to minimize any aftereffects of stimulation before proceeding to the second stimulation session. During this second thirty minutes of tACS, participants were stimulated either right frontally or left frontally, whichever they did not receive before. Simultaneously, they took a second version of the TTCT. Administration of left or right tACS was determined according to a double-blind study design. In addition to Cz, two electrodes were fixed to locations F3 and F4 for the entirety of the study so that the participants were not aware of the location which received stimulation. Both the order in which the TTCT versions were administered, as well as the order in which each hemisphere was stimulated, were randomized within each participant. Additionally, saliva samples for BDNF genotyping were collected and the handedness of each participant was recorded.

The TTCTs will be evaluated by a blinded third party, assigning numerical values to a creativity index and allowing for statistical, quantitative analysis of creativity performance and the correlation between creativity performance and left- versus right-frontal stimulation at the alpha frequency. We will report the unblinded results of this study at the meeting.

[1] Lustenberger, et al. (2015). Cortex, 67.
[2] Fink, et al. (2009). Hum. Brain Mapp., 30.

Disclosures: **B. Price:** None. **C. Lustenberger:** None. **S. Alagapan:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research

relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro, LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

506. Oscillations and Synchrony: Other I

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Topic: B.10. Network Interactions

Support: NARSAD Independent Investigator Award

Swiss National Science Foundation P300PA_164693

Title: Topography of alpha asymmetry in patients with major depressive disorder.

Authors: *S. A. UPADHYAYULA[1], S. ALAGAPAN[2], C. LUSTENBERGER[2], M. BOYLE[2], C. LUGO[2], J. MELLIN[2], M. ALEXANDER[2], D. RUBINOW[2], F. FROHLICH[2];
[1]Electrical Engin., North Carolina State Univ., Raleigh, NC; [2]Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Alpha (8 - 13 Hz) asymmetry, characterized by increased alpha power in left frontal electrodes compared to right frontal electrodes in resting state electroencephalograms (EEG), is widely reported in patients with mood disorders [1]. This asymmetry could serve as a potential target for non-invasive brain stimulation that modulates oscillations such as transcranial alternating current stimulation (tACS). We are currently conducting a clinical trial investigating the efficacy of tACS as a therapeutic for MDD patients. Here, we present preliminary results on the baseline topographical distribution of resting state alpha asymmetry in participants with major depressive disorder (MDD) and contrast it with healthy controls (HC). A total of 15 MDD (12 - Female) and 12 HC (9 - Female) participants were included in this analysis. The average Hamilton Rating Scale for Depression (HAMD) score of the MDD participants was 14.9 ± 4.5 (mean ± sd.). Eyes-open (EO) and eyes-closed (EC) 128 channel EEG was collected at the screening visit. The EEG preprocessing steps included line noise removal, robust average referencing and independent component analysis (ICA) to remove non-neural artefacts. Alpha band power spectral density (psd) was then computed using multi taper spectral analysis. Alpha asymmetry scores were computed as the difference in the log transformed psd between the homologous right (R) and Left (L) electrode pairs (ln(R) - ln(L)). We found a trend-level increase in the alpha asymmetry in the frontal regions [near F5/F9] in the eyes open condition in

the MDD population when compared to HC (-0.18 ± 0.01 vs. 0.02 ± 0.02, (mean ± s.e.m); p = 0.07 , two sample t-test). These results are in agreement with previously reported findings about the alpha asymmetry in the prefrontal regions of MDD patients. In contrast, alpha asymmetry was not different in the EC condition (-0.04 ± 0.01 vs. 0.05 ± 0.02; p = 0.24). These findings suggest that a more differentiated view of alpha asymmetry in MDD may emerge from such a more detailed topographic analysis. The correlation between HAMD score and alpha asymmetry was not significant, likely due to the small size of the population (r = -0.3, p = 0.26). Taken together, our results support frontal alpha asymmetry as a plausible physiological target and might serve as a marker of effectiveness for non-invasive therapeutic brain stimulation strategies.
[1] Davidson RJ. Brain Cogn. 1992 Sep;20(1):125-51.

Disclosures: **S.A. Upadhyayula:** None. **S. Alagapan:** None. **C. Lustenberger:** None. **M. Boyle:** None. **C. Lugo:** None. **J. Mellin:** None. **M. Alexander:** None. **D. Rubinow:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro, LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

506. Oscillations and Synchrony: Other I

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Support: NIH/NIMH Grant R21MH105557

Title: State-dependent entrainment by stimulation and the emergence of an asymmetric arnold tongue in a biophysical thalamic network model

Authors: *G. LI[1], C. HENRIQUEZ[2], F. FROHLICH[1];
[1]Psychiatry Dept., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Dept. of Biomed. Engin., Duke Univ., Durham, NC

Abstract: The thalamocortical system plays a central role in cerebral rhythmic oscillations and abnormal thalamocortical rhythms have been implicated in neurological and psychiatric disorders. Targeting brain oscillations with both invasive stimulation such as deep brain stimulation (DBS) and non-invasive brain stimulation such as transcranial magnetic stimulation

(TMS) have emerged as new promising therapeutic techniques that aim to restore physiological brain dynamics. However, a systematic understanding of how these stimulation paradigms interact with endogenous neural activity and neural oscillatory patterns to deliver therapeutic effects is currently lacking. Due to the critical role of the thalamus in mediating thalamocortical oscillations, we have developed a biophysically realistic thalamic network model that can generate multiple distinct states of oscillations (delta, spindle, alpha, beta and gamma oscillations) dependent on cholinergic and noradrenergic neuromodulation and afferent excitation. The successful manifestation of multiple distinct thalamic oscillations in one unified computational construct enabled us to systematically study the impact of stimulation on thalamic network dynamics. By employing a stimulation protocol consisting of brief current pulses, we examined the stimulation effects in a wide range of stimulation frequency and amplitude, and under four major oscillatory states (delta, alpha, beta and gamma). We found that (1) modulation of thalamic network oscillations critically depends on the endogenous frequency and (2) stimulation generates an asymmetric Arnold Tongue that favors stimulation frequencies higher than the endogenous frequency. The stimulation effect is also state-dependent such that lower frequency oscillations (delta and alpha) endowed with dynamics driven by intrinsic ion channels are more robust to stimulation perturbation than higher frequency oscillations (beta and gamma) that are predominantly driven by synaptic inputs. The interaction of intrinsic cellular dynamics and stimulation induced emergent network behaviors such as bistability and a discontinuity in entrainment as a function of the stimulation frequency. Furthermore, the model revealed that stimulation of the thalamic reticular nucleus (TRN) induced differential effects than stimulation of the lateral geniculate nucleus (LGN). Therefore, this biophysical modeling study offers important insights on the application of brain stimulation to the thalamus as a perturbation technique to target pathologically altered network dynamics associated neurological and psychiatric disorders.

Disclosures: **G. Li:** None. **C. Henriquez:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro, LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

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NC TraCS 2KR721505

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Swiss National Science Foundation

Title: Sleep spindles and cognitive social development in infants and toddlers

Authors: *J. PAGE[1], C. LUSTENBERGER[2], M. MURIAS[3], F. FROHLICH[2];
[1]Educ. and Psychology, [2]Psychiatry, Univ. of North Carolina At Chapel Hill, Chapel Hill, NC;
[3]Duke Inst. for Brain Sci., Duke Univ., Durham, NC

Abstract: A characteristic of early childhood is rapid development of cognitive capabilities and their underlying network-level substrate. Sleep is put forth as a means to assess these changes by examining the presence of sleep spindles. Sleep spindles are thalamo-cortically generated brain rhythms at 10-16 Hz in non-rapid eye movement (NREM) sleep [1] and are related to intelligence and general learning traits in adults and school aged children [2]. Sleep spindle characteristics are widely studied young adults, in older children (5 – 15 years old), and show clear developmental changes [3]. Furthermore, they are altered in several disorders, including autism spectrum disorder [4], schizophrenia, and neurodegenerative disorders. They may, therefore, represent a useful baseline biomarker for neurodevelopment to distinguish typical development from atypical development. However, the topographical and spectral characteristics of sleep spindles has yet to be examined in typically developing infants/toddlers 12 -30 months of age and moreover how these features are associated with outcomes of early cognitive development in this population.

To do address this gap, we are conducting a study on characterizing the topography of sleep spindles in children 12 – 30 months of age. Here we report preliminary nap data of a 26 month-old male with high density electroencephalogram (hdEEG, 128 electrodes). Initial findings depict clear spindle activity with two distinct spindle peaks, one around 12.5 Hz and one around 16 Hz during NREM sleep. In the topographical analysis, we found that the 16 Hz peak is most pronounced at parietal and frontal locations whereas the 12.5 Hz peak is most distinct at central locations.

This finding is in contrast to the topographical distribution of sleep spindles in young adults where slow spindles occur frontally, and fast spindles are detected centro-parietally. Furthermore, the highest frequency peak in adults is around 14 Hz [1,5] compared to 16 Hz that is reported here. Further research will investigate whether this spindle frequency is representative of individuals of this specific age (12 - 30 months), or particular to this child.

The long-term goal of this research is to establish if sleep spindles present a biomarker of typical development, which could then be used at an early age to assess risk for neurodevelopmental disorders.

[1] De Gennaro & Ferrara. (2003) Sleep Med. 7

[2] Lustenberger et al. (2012). PLoS One 7

[3] Doucette, et al. (2015). Brain Sci. 5
[4] Tessier, et al. (2015). Int J Psychophysiol, 97
[5] Andrillon (2011) The Journal of Neuroscience 20

Disclosures: **J. Page:** None. **C. Lustenberger:** None. **M. Murias:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock in EGI. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock Holder of Pulvinear Neuro, LLC. F. Consulting Fees (e.g., advisory boards); Strata Solar.

Poster

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Topic: B.10. Network Interactions

Support: UNC Department of Psychiatry

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Title: Cortical oscillations in juvenile ferrets following maternal immune activation during pregnancy

Authors: ***Y. LI**[1], J. H. GILMORE[1], F. FROHLICH[1,2,3,4,5];
[1]Dept. of Psychiatry, [2]Neurobio. Curriculum, [3]Dept. of Cell Biol. and Physiol., [4]Dept. of Biomed. Engin., [5]Neurosci. Ctr., Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: Abnormalities in neuronal synchronization and oscillations have been implicated in schizophrenia and other psychiatric disorders. Although studies suggested that decreased amplitude and synchrony of cortical oscillations in adolescence underlie the pathology of schizophrenia[1], it is still unknown when in the course of development these alterations arise. Here, we adapted an existing prenatal maternal-immune activation paradigm[2], which models features of schizophrenia, and applied it in the ferret. We injected poly I:C (10mg/kg, i.p.) into pregnant ferrets at E30 (full gestation ~41 days) and confirmed the occurrence of an immune activation by detecting an increase of body temperature. Multi-electrode arrays were implanted in the primary visual cortex in the offspring; spontaneous and light-evoked unit activity and local

field potential (LFP) were recorded before and after eye-opening in the freely moving animal. The results were compared to data from age-matched controls.
Overall, the spontaneous oscillation amplitude was increased after eye-opening compared to before eye-opening, especially in the higher frequency range (>30Hz). These gamma oscillations were decreased in amplitude in the poly I:C group compared to the control in both age groups ($p<0.001$, student-t test). Furthermore, phase-amplitude coupling of the gamma oscillation amplitude to the phase of lower frequency oscillations (delta, theta and alpha) was significantly decreased in the poly I:C group in both age groups ($p < 0.01$). Whole-field light flashes induced a peak in the gamma band of the LFP and the amplitude of this visual response was decreased in the poly I:C group ($p < 0.001$ for both age groups). Finally, in control animals, cortical oscillations were entrained by visual stimuli with frequency up to 30 Hz after eye-opening. This effect was decreased in the poly I:C group for higher frequencies (> 10 Hz).
Our results suggest that the functional interaction between neuronal circuits by synchronization of electrical activity patterns is impaired from very early stage in childhood development after environmental insults that increase risk for schizophrenia. A parallel study is investigating whether the maternal-immune activated ferret offspring exhibit behavioral changes consistent with abnormalities observed in schizophrenia. The long-term goal is to leverage these findings into novel brain stimulation treatments that aim to correct the observed aberrant neuronal activity pattern to restore physiological development of cortical circuits.
1. Uhlhaas and Singer (2011) Schizophr. Bull. 37: 514-523
2. Meyer et al. (2005) Neurosci. Biobehav. R. 29: 913-947

Disclosures: **Y. Li:** None. **J.H. Gilmore:** None. **F. Frohlich:** None.

Poster

506. Oscillations and Synchrony: Other I

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Topic: B.10. Network Interactions

Support: Tal Medical

Title: High-frequency electric field stimulation modulates neocortical network dynamics *In vitro*.

Authors: ***S. L. SCHMIDT**[1], E. NEGAHBANI[2], N. MISHAL[2], F. FROHLICH[2];
[1]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Univ. of North Carolina, Chapel Hill, NC

Abstract: Low field magnetic stimulation (LFMS) is a form of brain stimulation that applies small magnitude transient magnetic fields and has been shown to rapidly improve mood in patients with bipolar disorder and major depressive disorder [1]. The mechanism of action of LFMS is unknown. Here we applied an electric field with the same waveform as the induced electric field resulting from LFMS (0.5 kHz pulse train, patterned at 0.5 Hz) to acute cortical slices and compared the resulting network dynamics to sham stimulation (i.e., no electric field stimulation). We asked if such high-frequency electric field stimulation can induce outlasting changes to the network dynamics *in vitro*. We recorded multiunit (MU) spiking activity with a 6x10 array of microelectrodes from coronal sections of mouse prefrontal cortex. The brain slices were perfused with *in vivo*-like artificial cerebral spinal fluid with 5 μM carbachol to provide neuromodulatory drive and increase neuronal excitability. We compared multiunit firing rate (FR) and oscillatory power before and after 20 minutes of electric field stimulation (20 mV/mm peak to peak, verum) or sham stimulation. The presence of carbachol caused the firing rate to gradually increase in absence electric field stimulation as reported in [2]. During verum stimulation, FR increased less than during sham stimulation (increase in FR: 29.0% verum stimulation vs. 39.1% sham, n = 631 and 832 MU, 13 and 17 slices respectively, p = 0.013 Wilcoxon ranked sum). These experiments suggest that LFMS-type waveforms can reduce neuronal excitability and that these changes outlast the stimulation. We will extend this study to examine the effect of stimulation in absence of exogenous neuromodulators and also combined with other forms of neuromodulatory activation. A limitation that our study shares with other *in vitro* examinations of EF stimulation is that the amplitude used here is substantially higher than used for human stimulation [3]. Further studies are needed to understand the underlying mechanisms and to link these findings to the observed clinical effects of LFMS.
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Disclosures: S.L. Schmidt: None. **E. Negahbani:** None. **N. Mishal:** None. **F. Frohlich:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Funding from Tal Medical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Majority stock holder of Pulvinar Neuro, LLC.. F. Consulting Fees (e.g., advisory boards); Strata Solar, Travel Support Tal Medical.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 506.17/N12

Topic: B.10. Network Interactions

Support: NSF Graduate Research Fellowship

Sloan Research Fellowship

Title: Nonsinusoidal oscillatory shape can drive spurious cross-frequency coupling

Authors: *S. R. COLE[1], B. VOYTEK[2];
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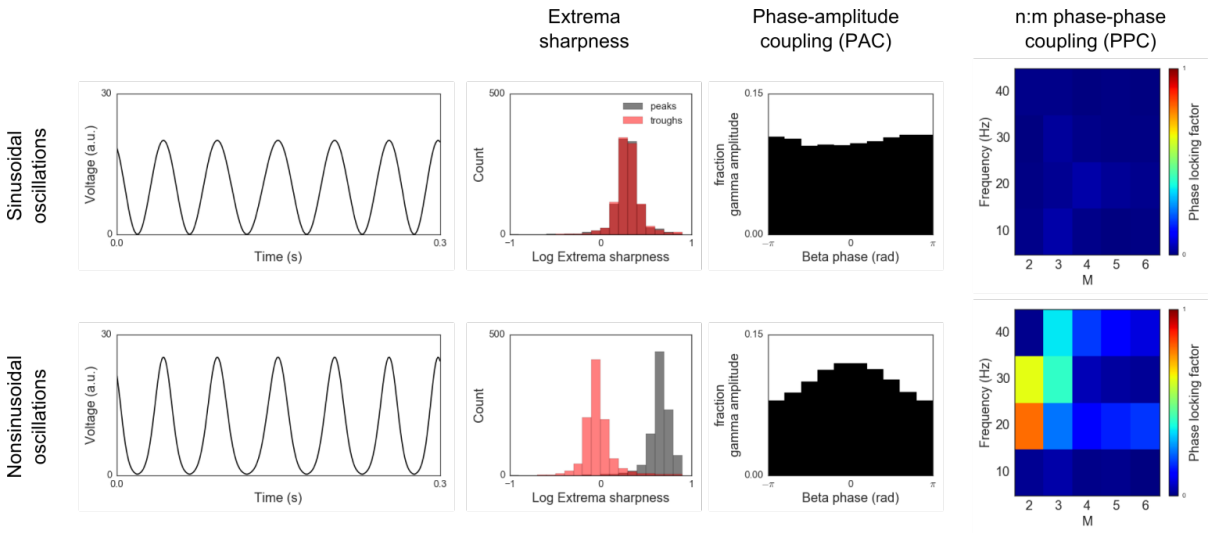
Abstract: Oscillatory activity is abundant in electrophysiology across species, brain regions, and spatial scales. Neural oscillations are thought to be critical for communication within and across brain structures, as supported by hundreds of publications that report cross-frequency coupling in electrophysiological signals.
While these analyses are based on the Fourier transform, a decomposition method that assumes a sinusoidal basis, numerous reports have shown neural oscillations that are nonsinusoidal. Therefore, we hypothesize that the temporal features of the shape of a nonsinusoidal oscillatory waveform, rather than sinusoidal spectral features, may underlie apparent cross-frequency coupling.
Here we simulate electrophysiological signals with different biophysically-plausible waveforms and show that the waveform shape biases measures of both phase-amplitude coupling (PAC) and n:m phase-phase coupling (PPC, see Figure). That a temporal domain feature arising from one rhythmic process can produce apparent PAC and PPC contrasts with the two distinct oscillatory components that are often assumed to underlie them.
We conclude that naive application of Fourier analysis to electrophysiology may lead to misleading interpretations stemming from the underappreciated fact that neural oscillations are nonsinusoidal. For this reason, we have developed alternative measures for analyzing time series data that are more parsimonious than frequency domain analyses that are biased by waveform shape. This temporal-domain approach is justified by reports showing that behavior, disease state, and neurotransmitter presence affect oscillatory shape. In conclusion, both spectral and temporal analyses may be necessary to extract, and interpret, information in electrophysiological signals.



Disclosures: **S.R. Cole:** None. **B. Voytek:** None.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 506.18/N13

Topic: B.10. Network Interactions

Title: Sparsely synchronized brain rhythms in an inhomogeneous small-world complex neuronal network

Authors: ***W. LIM**[1], S.-Y. KIM[2];
[1]Daegu Natl. Univ. of Educ., Daegu, Korea, Republic of; [2]Inst. for Computat. Neurosci., Daegu, Korea, Republic of

Abstract: We consider an inhomogeneous small-world network (SWN) composed of inhibitory short-range (SR) and long-range (LR) interneurons, and study emergence of sparsely synchronized rhythms by varying the fraction of LR interneurons p_{long} from 0 to 1. Although SR and LR interneurons have the same average in- and out-degrees (representing the potentiality in communication activity), their betweenness centralities (characterizing the potentiality in controlling communication between other interneurons) are distinctly different. Hence, in view of betweenness, SWNs we consider are inhomogeneous, unlike the "canonical" Watts-Strogatz SWN with nearly same betweenness centralities. For small p_{long}, the average betweenness centrality of LR interneurons is much larger than that of SR interneurons. Hence, the load of

communication traffic is much concentrated on a few LR interneurons. However, with further increase in p_{long} the number of LR connections (coming from LR interneurons) increases, and then the average betweenness centrality of LR interneurons decreases. Hence, as a result of increase in p_{long}, the average path length becomes shorter, and the load of communication traffic is less concentrated on LR interneurons, which leads to better efficiency of communication between interneurons. Particularly, we investigate the effect of network topology (e.g., average path length and betweenness) on sparsely synchronized rhythms. When passing a small critical value $p^{(c)}_{long}$ ($\simeq$ 0.16), sparsely synchronized rhythms are found to emerge. This transition from desynchronization to sparse synchronization is well characterized in terms of a realistic thermodynamic order parameter. For $p_{long} > p^{(c)}_{long}$, sparse synchronization occurs in the whole population because the spatial correlation length between neuronal pairs covers the whole system, thanks to sufficient number of LR connections. The degree of sparse synchronization is also well measured in terms of a realistic "statistical-mechanical" spiking measure, and an optimal sparse synchronization is found to occur at a dynamical-efficiency optimal value $p^{(o)}_{long}$ ($\simeq$ 0.27) through trade-off between synchronization and axon wiring cost. For this optimal case, responses to external time-periodic stimulations (applied to sub-groups of LR and SR interneurons, respectively) are studied and discussed in relation to the betweenness, representing the effectiveness of information transfer in the whole network.

Disclosures: **W. Lim:** None. **S. Kim:** None.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

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Program#/Poster#: 506.19/N14

Topic: B.10. Network Interactions

Title: Competence of neuronal network models with random couplings to support slow oscillations

Authors: ***C. A. FUNG**, T. FUKAI;
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Abstract: Slow oscillations (~1Hz) are observed in mammals' brains during sleeping or resting, which are also believed to be related to memory consolidation during non-rapid-eye-movement sleep. There are reports showing that removal or inactivation of the thalamus will not completely impair the occurrence of UP states, although the occurrence of UP states are found to be contributed by inputs from the thalamus (Crunelli and Hughes, 2010). This observation suggested that local networks may have the ability to support UP state for at least a transient

period, so that UP states may still occur without input from the thalamus. To understand the local dynamics enabling the transient sustainability of UP states, in this work, we have studied networks of spiking neurons with randomly-distributed couplings. By using mean-field analysis, condition of parameters to support sustainable UP states and transient UP states are obtained, which is further verified by series of simulations. We have also studied how network behaviors differ between networks with log-normal distributed couplings and sparse-Gaussian couplings. Statistical analysis of those network behaviors are also presented.

Disclosures: **C.A. Fung:** None. **T. Fukai:** None.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 506.20/N15

Topic: B.10. Network Interactions

Support: R03MH103728

R01MH085074

Title: Spontaneous intracellular membrane voltage fluctuations in cortical pyramidal cells are inconsistent with balanced excitation and inhibition

Authors: ***F. R. FERNANDEZ**, J. A. WHITE;
Biomed. Engin., Boston Univ., Boston, MA

Abstract: Analyses of cortical neuron intracellular membrane voltage fluctuations resulting from sensory evoked activity, as well as up-down state transitions observed in anaesthetized rodents, indicates that these events are generated through a joint increase in excitatory and inhibitory currents. Accordingly, an increase in excitation is quickly followed and matched with an increase in inhibition. This form of balanced and correlated excitation and inhibition results from feedforward- and feedback-inhibition present in cortical circuits. It remains unclear, however, if spontaneous voltage fluctuations under awake conditions are also the product of balanced and temporally correlated excitatory and inhibitory synaptic activity. Unlike sensory evoked depolarizations or up-states, spontaneous voltage fluctuations lack an external reference point from which to measure changes in currents and calculate synaptic conductances. Nevertheless, if balanced and temporally correlated synaptic activity underlie spontaneous fluctuations, we would expect that mutual cancelation of excitatory and inhibitory events is maximal near the net synaptic reversal potential. For this reason, the relationship between the standard deviation of

membrane voltage fluctuations and the mean holding voltage should be non-monotonic; fluctuations are smallest near the net synaptic reversal potential but grow larger at voltages above or below the net synaptic reversal value. To test this hypothesis, we carried out visually-guided intracellular patch-clamp recordings of layer II somatosensory neurons in awake, head-fixed mice using 2-photon microscopy. Using tdTomato expression in CaMK2-postive neurons, we targeted pyramidal cells and carried out recordings in both current- and voltage-clamp across a range of subthreshold voltage values. Contrary to predictions from models of balanced and correlated synaptic activity, the standard deviation and power of fluctuations increases monotonically with depolarization from -90 to -40 mV. Further analysis indicates that spontaneous voltage fluctuations are largely the product of excitatory synaptic currents that are amplified by a voltage-dependent increase in pyramidal cell membrane input resistance.

Disclosures: F.R. Fernandez: None. **J.A. White:** None.

Poster

506. Oscillations and Synchrony: Other I

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Program#/Poster#: 506.21/N16

Topic: B.10. Network Interactions

Support: NSF GRFP: DGE 1256260

NSF PoLS 1058034

NIH NIBIB EB018297

Rackham Merit Fellowship

Title: Spike frequency adaptation and memory selectivity

Authors: *J. P. ROACH[1,2], L. M. SANDER[3], M. R. ZOCHOWSKI[3];
[2]Neurosci. Grad. Program, [3]Physics, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: In the brain, representations of the external world are encoded by patterns of neural activity. It is critical that representations be stable so that they are easily reproduced. On the other hand there are times when representations need to be rapidly moved between. In this work we point to the effects of the neurotransmitter acetylcholine (ACh) is a plausible mechanism for the contextual stabilization (and destabilization) of neuronal representations. Acting through the muscarinic system ACh regulates the magnitude of spike frequency adaptation (SFA) by deactivating slow potassium currents. We use two computational models to show that the

magnitude of SFA exhibited by neurons selects for representations that have been stored within synaptic weights at different strengths. For low levels of SFA (which correspond to high levels of ACh), networks will stabilize in a representation that most closely corresponds to the initial network state. Increasing levels of SFA, by decreasing modeled ACh, leads to only the patterns corresponding to the strongest memories being stable. We show in a modified Hopfield network that increased SFA destabilize representations in a manner similar to fast, or thermodynamic noise, but also allows for deterministic, periodic activation of representations through time. Additionally, we demonstrate that SFA modulation controls selectivity for spatially localized representations in a biophysical model of cholinergic modulation in cortical networks. This model produces localized bumps of firing. A region with enhanced recurrent excitation stabilizes the bump location as a spatial representation and selectivity for these regions is quickly diminishes as SFA levels increase. When multiple spatial representations of varying strengths are stored in a network moderate increases of SFA level lead to strength dependent destabilization of representations in a quantitatively similar manner to the Hopfield model. We argue that control of SFA level is a universal mechanism for network-wide memory selectivity and that SFA provides a biologically plausible mechanism for switching between stable representations in cortical networks.

Disclosures: **J.P. Roach:** None. **L.M. Sander:** None. **M.R. Zochowski:** None.

Poster

506. Oscillations and Synchrony: Other I

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Program#/Poster#: 506.22/N17

Topic: B.10. Network Interactions

Support: NSERC

AIHS

CFI

IAE

Title: Glucose modulation of synchronized network oscillations in ventromedial hypothalamic nucleus of acute and organotypic newborn rat brain slices

Authors: B. RAWAL, V. RANCIC, A. SU, *K. BALLANYI;
Physiol., Univ. Alberta, Edmonton, AB, Canada

Abstract: Glucose-sensitive neurons comprise neural networks in the ventromedial hypothalamic nucleus (VMN). Here we studied in 300 μm thick coronal slices from 0-3 days old rats whether neonatal VMN neurons (i) are already glucose-sensitive, (ii) show early network oscillations (ENOs) like neurons in immature neonatal brain regions (iii) retain their properties under 'organotypic' culture conditions. Acute slices showed a rhythmic field potential (single event duration 12-15 s, rate 4-5 bursts/min). Whole-cell recording combined with cytosolic calcium imaging using the fluorescent dye Fluo-4 revealed the field potential is due to synchronized rhythmic action potential discharge of VMN neurons causing calcium rises. Bolus-loading with Fluo-4 showed that >80% of fluorescent cells are rhythmic. In slices generated and kept in 3 mM glucose, lowering glucose to 1 mM accelerated the rate of electrical and calcium oscillations and 10 mM glucose slowed both events by 15-20%. VMN cells in cultured slices did neither change their morphology nor their responses to neuromodulators like ATP or glutamate for up to six weeks. Synchronous oscillations like in acute slices emerged after one week of culturing and were stable (and glucose-sensitive) between weeks 2-6. Our data indicate that (i) glucose-sensitive neurons regulate ENO-like oscillations in which the majority of neonatal VMN cells are involved and (ii) cultured VMN slices are a potent model for studying long-term pharmacological or genetic modulation of neural network activity.

Disclosures: **B. Rawal:** None. **V. Rancic:** None. **A. Su:** None. **K. Ballanyi:** None.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

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Program#/Poster#: 506.23/N18

Topic: B.10. Network Interactions

Support: NIH NIBIB 1R01EB018297

NSF PoLS 1058034

Title: Synchronous bursting properties of interneuron networks are affected by cholinergic modulation of intrinsic cellular properties

Authors: *S. RICH[1], V. BOOTH[2], M. ZOCHOWSKI[3];
[1]Mathematics, [2]Mathematics and Anesthesiol., [3]Physics and Biophysics, Univ. of Michigan, Ann Arbor, MI

Abstract: Acetylcholine (ACh) modulates cell excitability via the slow, M-type potassium current (M-current). There is evidence that some of the PV interneurons, present in the

hippocampus and cortex, contain a M-current and are reciprocally connected. The OLM interneurons of the hippocampus also contain a M-current, although the presence of reciprocal connections is less clear. A proportion of cortical pyramidal cells also contain a M-current. The dynamics of the M-current affect cellular properties, typified through the cell's IF (current-frequency) curve and PRC (phase response curve), which quantifies the change in phase of the subsequent firing of a neuron in response to stimuli at various phases within its periodic firing cycle. In the absence of ACh, the M-current is active and the neuron exhibits Type II properties, including a shallow IF curve with a minimum firing frequency and a PRC showing a phase delay in response to an excitatory pulse soon after firing. An active M-current also causes spike-frequency adaptation (SFA), leading to an increased firing rate if the cell has not fired in the recent past. However, ACh blocks the M-current, causing the neuron to exhibit Type I properties including a steep IF curve with repetitive firing at arbitrarily low frequencies and a PRC that always shows phase advance. Our research shows that the changes in cellular properties caused by ACh affect an interneuron network's tendency to exhibit synchronous bursts of activity, be the network comprised strictly of inhibitory interneurons or of excitatory and inhibitory cells in a coupled, excitatory-inhibitory (E-I) network. Simulated networks of randomly connected inhibitory Type I neurons exhibit bursting activity via one-cluster dynamics in which largely the same neurons participate in each burst; however, simulations containing Type II neurons with an active M-current either exhibit one-cluster dynamics or a distinct two-cluster dynamic in which subsequent bursts of network activity contain mutually exclusive neuronal populations. The observed clustering dynamic is dependent upon the duration of synaptic inhibition and the magnitude of the external drive to the network. This behavior is distinct from the dynamics of networks of neurons with Type II properties but lacking a M-current or SFA. Analysis of E-I networks reveals that cholinergic modulation of the M-current causes significant changes in the rhythmic dynamics of the network. When properties of the visual cortex motivate the neuronal makeup of the E-I network, the changes caused by ACh may elucidate the mechanisms underlying some of the observed behavioral effects of cholinergic modulation on this system.

Disclosures: **S. Rich:** None. **V. Booth:** None. **M. Zochowski:** None.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 506.24/O1

Topic: B.10. Network Interactions

Support: MRC studentship

Title: The role of Parvalbumin and Somatostatin interneurons in hippocampal gamma oscllations

Authors: *P. ANTONOUDIOU, A. L. UPTON, E. O. MANN;
Univ. of Oxford, Oxford, United Kingdom

Abstract: The generation and long-range-synchronisation of cortical network oscillations at gamma (γ)-frequency (30-100 Hz) appears to correlate with specific cognitive functions. In order to resolve how rhythmic and/or synchronous patterns of activity during γ-oscillations contribute to cortical circuit computations, it is necessary to understand the mechanisms underlying their generation. There is a consensus that these rhythms depend on the spiking of inhibitory interneurons, which synchronise the firing of excitatory pyramidal cells via fast synaptic inhibition. Inhibitory interneurons constitute a heterogeneous group of neurons that can be distinguished based on their expression of neuropeptides, calcium binding proteins, and the subcellular targets of their axonal projections. It has previously been shown that the parvalbumin-positive (PV+) interneurons, which target the perisomatic domain of pyramidal neurons, play a key role in generating and maintaining γ-oscillations in the brain. However, as far as we are aware, it has not been tested how direct activation of another major interneuron class, the somatostatin-positive (SST+) interneuron, affects the generation of rhythmic network activities. Therefore, we stereotaxically injected PV-CRE and SST-CRE mice in the ventral hippocampus with a CRE-dependent AAV5 vector that carried the light sensitive cation channel, Channelrhodopsin 2 (ChR2). This enabled us to examine how activation of PV+ or SST+ interneurons affects network activity in the CA3 of acute hippocampal slices. Light activation of either PV+INs or SST+INs at 40 Hz could entrain cholinergically-induced (5 μM carbachol) γ-oscillations in acute hippocampal brain slices. However, continuous activation of PV+INs suppressed ongoing γ-oscillations, whereas activation of SST+INs was able to induce γ-oscillations even in the absence of cholinergic drive. These results suggest that there may be more than one type of cortical microcircuit that generates γ-oscillations.

Disclosures: P. Antonoudiou: None. **A.L. Upton:** None. **E.O. Mann:** None.

Poster

506. Oscillations and Synchrony: Other I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 506.25/O2

Topic: B.10. Network Interactions

Support: ERC (SEMBIND)

Title: Theta and delta rhythms underlie cross-regional interactions during semantic processing

Authors: *N. ADAMS[1], C. TEIGE[2], G. MOLLO[2], T. KARAPANAGIOTIDIS[2], P. L. CORNELISSEN[4], J. SMALLWOOD[2], M. A. WHITTINGTON[3], B. JEFFERIES[2]; [1]Biol., [2]Psychology, [3]HYMS, Univ. of York, York, United Kingdom; [4]Psychology, Northumbria Univ., Newcastle upon Tyne, United Kingdom

Abstract: Objectives: To investigate spatio-temporal aspects of cross-regional dynamics amongst brain areas involved in semantic processing.
Methods & Results: 17 participants performed a semantic task in which two words were presented in succession (these were either strongly or weakly related or unrelated). They decided if the two words were linked in meaning and were cued to make an overt response on occasional catch trials. Response times were also assessed for the same task outside the scanner. Data were beamformed following co-registration to the individuals' structural MR scan to give 7 nodes of interest in anterior, posterior and temporal cortices. All analyses were performed in MATLAB. Following stimulus delivery a clear trajectory of cross-covariant pairs was seen. However, strongly and weakly related word pairs generated differing trajectories resulting in co-activation of different node clusters immediately prior to when a response was expected. Highly synchronous activity was seen in the node triplet MFG-MTG-PCC prior to response in the strong association condition. In contrast a quadruplet of highly synchronous node pairs (ATL-AG-PCC-MTG) was seen prior to response in the weak association condition. Difference spectrograms for strong-vs-weak association trials revealed a theta-to-delta frequency inverse-chirp signal, with the response time corresponding to the lower bound of this event.
Conclusions: Although averaged individual regional responses to the two task conditions were indistinguishable, the resulting cross-regional interactions were both temporally and structurally different depending on the associative strength between semantically-related words.

Disclosures: N. Adams: None. **C. Teige:** None. **G. Mollo:** None. **T. Karapanagiotidis:** None. **P.L. Cornelissen:** None. **J. Smallwood:** None. **M.A. Whittington:** None. **B. Jefferies:** None.

Poster

506. Oscillations and Synchrony: Other I

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 506.26/O3

Topic: B.10. Network Interactions

Support: ERA-NET EuroTransBio9: In-HEALTH; BMBF #031B0010B

Title: Striatal and cortical networks on microelectrode arrays - an *In vitro* model for Hepatic Encephalopathy

Authors: *S. THEISS[1], A. SCHNITZLER[1], O. A. SERGEEVA[2,1], W. FLEISCHER[1];
[1]Inst. of Clin. Neurosci., [2]Inst. of Neurophysiol., Univ. of Duesseldorf, Duesseldorf, Germany

Abstract: Hepatic encephalopathy (HE) is associated with pathologically raised ammonia and glutamine levels. HE patients present with severe but potentially reversible neurological symptoms, while functional imaging suggests basal ganglia involvement. Recent studies showed that oxidative stress in hyperammonemic animals was especially severe in somatosensory cortex. In order to establish a functional brain region specific *in vitro* model for HE, P0 rat coronal corticostriatal slices were processed to prepare parallel primary dissociated cultures of striatum or cortex, grown separately on microelectrode arrays (MEAs) with 60 Ti/TiN electrodes (30 µm diameter, 200 µm spacing; Multichannel Systems). Cultured striatal neurons widely expressed glutamate decarboxylase and vesicular GABA transporter, but only low levels of vesicular glutamate transporters VGLUT 1/2 (2—4% of cortical culture levels). Mature cortical and striatal cultures (>21 DIV) on MEAs showed spontaneous synchronous network bursts with distinct responses to elevated levels of ammonium or glutamine applied for 10 minutes. Ammonium (5 mM) increased burst rate of cortical neurons 1.3-fold, but inhibited bursting in striatal neurons 0.75-fold. In striatal cultures, glutamine (2 mM) increased spike rate 3.5-fold and burst rate 7.2-fold. Glutamine action was weaker in cortical cultures: spike and burst rates were increased 1.3-fold and 5-fold, respectively. In both striatal and cortical cultures, glutamine evoked a long-lasting stable network state with more and shorter bursts and more spikes outside of bursts that persisted over 20 minutes post washout. In Ca^{2+} imaging experiments, striatal cultures showed a glutamine-induced intracellular Ca^{2+} rise that was independent of neuronal activity (persisted in TTX), but could be blocked by competitive inhibition of system A amino acid transport (MeAiB), as well as by ionotropic glutamate receptor antagonists, suggesting a glutamate-mediated mechanism. Glutamate release from striatal cells could indeed be verified with an enzymatic assay kit. Thus, extracellular glutamine was taken up by neurons and triggered release of glutamate, which astrocytes then converted to glutamine and released back into extracellular space—a feedback-loop causing sustained long-lasting excitation of network activity after termination of drug application. Therefore, glutamine may play an important role in striatum-related symptoms of HE. In line with results from *in vivo* studies on HE models, this MEA-based *in vitro* approach revealed tissue-specific responses to ammonium and glutamine.

Disclosures: S. Theiss: None. **A. Schnitzler:** None. **O.A. Sergeeva:** None. **W. Fleischer:** None.

Poster

507. Oscillations and Synchrony: Other II

Location: Halls B-H

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Program#/Poster#: 507.01/O4

Topic: B.10. Network Interactions

Support: DARPA W911NF-14-2-0043

Title: Intrinsic network for mood in the human brain

Authors: *L. A. KIRKBY[1], F. LUONGO[1], M. NAHUM[2], T. VAN VLEET[2], M. LEE[1], H. DAWES[1], E. CHANG[1], V. SOHAL[1];
[1]UC San Francisco, San Francisco, CA; [2]Posit Sci., San Francisco, CA

Abstract: Mood is an inherent part of what makes us human, however the neural circuits underlying mood regulation are poorly understood. Here, we used chronic, large-scale electrocorticography (ECoG) recordings from the human limbic system to identify sub-networks relevant to mood. Recordings were collected from patients with epilepsy undergoing surgical evaluation, over a period of several days. We assessed functional interactions between different brain regions by computing signal coherence across recording sites, from which we constructed a time series of coherence matrices in various frequency bands. We used independent components analysis to identify intrinsic connectivity networks (ICNs) within our datasets. Each dataset contained on the order of ten ICNs per frequency band, with some redundancy across bands. We postulated that sub-networks extracted in this way could be relevant to mood processing. Patients' psychological state was assessed using Posit Science's mood tracking application at several time points over the course of several days. Using a regression analysis, we found that activity patterns represented by a single ICN consistently correlated with mood state across patients. We identified the components that comprise this mood-correlated ICN to determine a neural representation for mood in the human brain. These findings have implications towards potential treatment for mood disorders, including anxiety and depression.
These data are part of the SUBNETS program, a collaborative project investigating circuit function and dysfunction in neuropsychiatric conditions.

Disclosures: L.A. Kirkby: None. **F. Luongo:** None. **M. Nahum:** None. **T. Van Vleet:** None. **M. Lee:** None. **H. Dawes:** None. **E. Chang:** None. **V. Sohal:** None.

Poster

507. Oscillations and Synchrony: Other II

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Program#/Poster#: 507.02/O5

Topic: B.10. Network Interactions

Support: DFG grant (SFB936/A3/B6)

EU grant (ERC-2010-AdG-269716)

Title: Attentional modulations of neuronal interactions during multisensory processing: An MEG study employing a visuotactile matching paradigm.

Authors: *F. GÖSCHL[1], U. FRIESE[1], J. DAUME[1], P. KÖNIG[2,1], A. K. ENGEL[1];
[1]Dept. of Neurophysiol. and Pathophysiology, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [2]Inst. of Cognitive Sci., Univ. of Osnabrück, Osnabrück, Germany

Abstract: Attentional selection of sensory signals and their integration across modalities require flexible interaction between remote brain areas. One candidate mechanism to establish local and long-range communication in the brain and thereby also address the challenge of binding stimulus features across modalities is transient synchronization of neural assemblies. In the present study, we used magnetoencephalography (MEG) to record brain activity from healthy human participants engaged in a pattern matching paradigm requiring the evaluation of concurrently presented visual and tactile stimuli. To investigate the interplay of bottom-up stimulus processing and top-down demands, we manipulated the crossmodal relation of the two stimuli as well as the focus of spatial attention. Spectral power in the theta- (2-7 Hz), alpha- (7-13 Hz), beta- (13-30 Hz) and gamma-bands (60-90 Hz) was analyzed on the sensor level and projected to the level of cortical sources using beamforming. We found neuronal activity in anticipation of crossmodal stimulation in the alpha- and beta-bands to be modulated by the direction of attention. Oscillatory power was reduced in cortical areas contralateral to the attended side, the maxima being located in higher visual areas, somatosensory association cortex, and supramarginal gyrus. For the processing of crossmodal information, attentional modulations were reflected in the beta- and gamma-bands and located in cortical areas largely overlapping with those observed in the baseline contrasts. In contrast, differences in spectral power related to visuotactile stimulus congruence were only apparent in beta frequencies and maximal in premotor cortices. Additional analysis of the phase dynamics of oscillatory signals suggested synchronization in low-frequency activity to be critically involved in the deployment of multisensory attention. Taken together, these results provide further evidence that oscillatory synchronization is functionally relevant for communication in distributed brain networks, and suggest that the integration of visuotactile information depends on both, bottom-up and top-down factors.

Disclosures: F. Göschl: None. **U. Friese:** None. **J. Daume:** None. **P. König:** None. **A.K. Engel:** None.

Poster

507. Oscillations and Synchrony: Other II

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Program#/Poster#: 507.03/O6

Topic: B.10. Network Interactions

Support: CIHR MOP-12675

Human Frontier Science Program

Title: Mesoscale, *In vivo*, functional voltage neuroimaging metrics for the assessment of normal and pathological brain function in mouse models of psychiatric disease

Authors: *A. W. CHAN, J. M. LEDUE, Y. WANG, T. H. MURPHY;
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Abstract: Increasingly, functional neuroimaging, principally using fMRI, is being applied to the investigation of psychiatric and neurodegenerative disease to reveal underlying long-distance network pathologies in brain function and the potential discovery of novel biomarkers of disease and disease progression. We propose that intermediate, mesoscale, neuroimaging approaches, using modalities that more directly reflect neuronal activity, in mouse models of psychiatric disease, presents an important parallel and complementary approach that can reveal much about disease pathology not possible in human imaging paradigms alone and may provide a platform for testing therapeutic interventions. Imperative to the interpretation of these approaches is the demonstration of robust functional neuroimaging metrics by which to assess abnormal, mesoscale, cortical activity. We used high-speed (150 Hz), wide-field (8.5x8.5 mm), voltage-sensitive dye (RH1692) imaging to examine spontaneous and sensory-evoked (somatosensory, visual, and auditory) networks of cortical activity in head-fixed awake and isoflurane-anesthetized C57Bl6 mice. Voltage sensitive dyes are advantageous for comparing mutant mouse lines that model human psychiatric disorders because they do not require breeding to introduce activity sensors that may alter disease characteristics. We demonstrate that infraslow (<0.1 Hz) and slow (0.5-6.0 Hz) correlated spontaneous activity can recapitulate analogs of human long-distance resting-state networks (Chan et al. 2015 Nature Comm). Intrahemispheric cortical connectivity revealed highest connectivity (R values >0.8) within medial medial cortical structures including cingulate, motor cortices, and parietal association cortex. Using resting state analysis we define standard deviation maps of regional activity in different frequency bands as an overall measure of activity while regional correlation reflects synchrony within networks. Interestingly, regional values of standard deviation reflecting baseline activity were highest in the same structures exhibiting greatest synchrony. In addition, our approach also allows for analyses of fast cortical dynamics. We assessed input-output strengths and variance of peripherally-evoked cortical sensory activity as well as the cortical receptive field sizes

associated with primary and secondary sensory cortical activation. Our intention is to use these approaches to evaluate leading models of psychiatric disorders related to autism spectrum disorder using the SHANK3 mutant mouse lines.

Disclosures: **A.W. Chan:** None. **J.M. LeDue:** None. **Y. Wang:** None. **T.H. Murphy:** None.

Poster

507. Oscillations and Synchrony: Other II

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Program#/Poster#: 507.04/O7

Topic: B.10. Network Interactions

Support: DHHS/ NIH/ NIMH, NINDS, NEI/ IRP

Title: Resting-state fMRI signals in the macaque are differentially altered by transient inactivation of specific basal forebrain subregions.

Authors: ***J. N. TURCHI**[1], C. CHANG[2], F. Q. YE[3], B. E. RUSS[4], D. K. YU[3], J. H. DUYN[2], D. A. LEOPOLD[4,3];
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Abstract: The physiological origins of spontaneous fluctuations in functional MRI (fMRI) signals remain to be elucidated. One structure that may drive a component of spatially correlated spontaneous activity is the basal forebrain (BF), which provides significant input to widespread cortical areas. Here, we hypothesized that selective unilateral inactivation of BF subdivisions would affect spontaneous activity primarily in regions to which these subdivisions project, while spontaneous activity in the fields to which the other basal forebrain subregions project would remain unaltered. To investigate the contributions of different basal forebrain subdivisions, we measured changes in spontaneous fMRI fluctuations after reversibly inactivating specific BF regions. On separate dates, muscimol (18 mM-44mM, 1.8 - 2.5 µl/ site) was infused unilaterally into different portions of the BF in monkeys: Ch4al, Ch4am, and Ch2 (for a total of 22 infusions in 2 monkeys). For functional MRI, intravenous MION was administered and 3-5 resting-state scans, each lasting thirty minutes, were collected (EPI, 1.5 mm isotropic, TR = 2.5 s). Data from each scan were spatially co-registered to a single reference EPI scan, and fMRI time series were converted to units of percent signal change. We examined hemispheric differences in fMRI signal correlations after inactivating each of the above three BF subregions and found that across all targeted sites, correlated signal fluctuations and local signal amplitude were attenuated in

areas ipsilateral to the inactivation. The greatest attenuation in fMRI amplitude was localized to regions receiving projections from the inactivated BF subregion. Specifically, shared spontaneous fluctuations were attenuated in much of cortex ipsilateral to Ch4al injections (including visual, ventral posterior parietal, and insula), while the hippocampus and cingulate cortex were largely spared. This pattern showed high correspondence (hemisphere reversal) across the separate left- and right-hemisphere inactivation sessions. In contrast, inactivation of Ch4am mainly affected medial parietal and cingulate cortices, while the effects of Ch2 inactivation were observed primarily in hippocampus and cingulate. These results demonstrate the strong contribution of the basal forebrain in shaping spontaneous fMRI fluctuations, as well as their inter-area correlations.

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Poster

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Topic: B.10. Network Interactions

Support: National Science Centre (NCS DEC-2011/03/B/NZ4/03053)

Wellcome Trust (WT098352MA)

Title: The ventral tegmental area is involved in the generation of high frequency oscillations in the nmda receptor antagonist model of schizophrenia

Authors: ***M. HUNT**[1], M. OLSZEWSKI[2], J. PIASECKA[2], M. WHITTINGTON[1], S. KASICKI[2];
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Abstract: NMDA receptor antagonists are widely used to model some of the symptoms of schizophrenia. The pathophysiology of this disease remains unclear, however, certain brain regions including the nucleus accumbens (NAc) have been widely implicated in the disease. Systemic injection of NMDA receptors antagonists, such as MK801, are associated with the presence of high frequency oscillations (HFO, 130-180 Hz) which can be recorded in local field potentials from many brain regions, including the NAc. We have shown previously that reversible inhibition of the NAc by local infusion of tetrodotoxin (TTX) reduces the amplitude of MK801-enhanced HFO indicating that this oscillation involved the local NAc network.

Importantly, afferent regions powerfully modulate the activity of NAc neurons. However, it is not known to what extent HFO in the NAc may be driven by its afferent projections (ventral hippocampus, basolateral amygdala, prefrontal cortex and the ventral tegmental area). In this study, rats were implanted with electrodes in the NAc and guides targeted at these afferent sites. We found that unilateral infusion of TTX (1 ng in 1 μl) to the ventral tegmental area reduced the power of MK801 (0.15 mg/kg)-enhanced HFO on the ipsilateral but not contralateral side. Infusion of TTX (1 ng in 1 μl) to the prefrontal cortex or ventral hippocampus had negligible effect MK801-enhanced HFO, although TTX infusion to the amygdala produced a much weaker reduction in HFO power. These findings indicate that the ventral tegmental area is an important brain regions generating HFO in the NAc (and likely other regions) after systemic injection of NMDA receptor antagonists.

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Poster

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Topic: B.10. Network Interactions

Support: NIH Grant R01-GM056398

Title: State repertoire and self-organized criticality of mesoscopic cortical dynamics are not altered during anesthetic-induced unconsciousness

Authors: *A. G. HUDETZ[1], J. VIZUETE[2], S. PILLAY[3], G. A. MASHOUR[1];
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Abstract: Anesthesia may modify consciousness by altering information integration in cortical circuits. Consciousness has been linked to the repertoire of brain states and to self-organized criticality. Cortical neuronal populations form synchronized ensembles whose characteristics are state-dependent but this has not been rigorously tested in anesthesia. Spontaneous neuronal activity was recorded with 64-contact microelectrode arrays in visual cortex of chronically instrumented, unrestrained rats under stepwise decreasing levels of anesthesia with desflurane. In each condition, local field potentials (LFP) were recorded for 10 minutes, sampled at 1000 Hz and band-pass filtered at 4-60 Hz. The negative peaks of LFP exceeding mean minus 3 standard deviations (nLFPs) were detected at 10ms time bins and their spatial pattern was analyzed. The

nLFPs formed compact, spatially contiguous activity patterns (CAPs) with short lifetimes (~10ms). The frequency distribution of CAP sizes followed a power-law with slope -1.5 in deep anesthesia (8% and 6% desflurane) suggesting the presence of self-organized criticality. The distribution deviated slightly from power-law as the animals regained consciousness. Randomizing the LFP data destroyed the power-law. The average number of CAPs was elevated in both wakefulness (0% desflurane) and deep anesthesia (8% desflurane) associated with burst-suppression. The entropy of CAP sizes was highest in wakefulness but changed insignificantly at desflurane concentrations between 6% and 4% desflurane associated with the transition from unconsciousness to consciousness as implied by the return of the rats' righting reflex. The types of recurring CAPs categorized by K-means clustering were conserved at all anesthesia levels and wakefulness, although proportion of various types did change in a concentration-dependent manner. The results yield new knowledge about the dynamic landscape of ongoing population activity in rat visual cortex at graded levels of anesthesia. Neither the repertoire nor self-organized criticality of local population activity can account for anesthetic suppression of consciousness suggesting that the latter may depend more on large-scale or temporally longer-scale dynamics or changes outside of primary sensory cortex.

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Poster

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Support: VA CDA Award BX002130 (JMM)

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JTM received partial salary compensation and funding from Merck MISP, but has no conflict of interest with this work.

Title: Elicitation and rescue of schizophrenia-like gamma band abnormalities via optogenetic and pharmacological manipulation of cortical excitatory/inhibitory balance

Authors: *J. M. MCNALLY[1], S. THANKACHAN[1], J. T. MCKENNA[1], R. W. MCCARLEY[2], R. E. BROWN[2];
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Abstract: Abnormalities in gamma band (30-80 Hz) oscillations (**GBO**) are an important feature of schizophrenia (**Sz**), which have been linked to all symptom classes. Current models suggest that impaired NMDA receptor activity, particularly in certain populations of cortical interneurons, alters the delicate balance in activity between the inhibitory and excitatory components of the cortical circuit (**E/I balance**), principally through a reduction of the activity of interneurons which express parvalbumin (**PV**), thereby causing GBO abnormalities. Numerous model systems, such as administration of sub-anesthetic doses of NMDA antagonists (e.g. ketamine), effectively model symptoms of Sz in humans and elicit Sz-like GBO and behavioral phenotypes reminiscent of Sz in animals. However, novel methods to restore E/I balance and Sz-related behavioral changes are needed. Further, it would be useful to have a non-pharmacological method to elicit Sz-like GBO abnormalities and correlate these abnormalities with behavior.

Here, utilizing both optogenetic and pharmacological techniques, we investigated whether: (i) Increased activity of cortically projecting basal forebrain (**BF**) GABAergic PV neurons, which target cortical PV interneurons and modulate cortical GBO (Kim et al., 2015, *PNAS* 112(11):3535), would mimic Sz-like increases in spontaneous GBO and locomotor activity; and (ii) Whether optogenetic inhibition of BF PV neurons or manipulation of the type 5 metabotropic glutamate receptor (**mGluR5**), which is expressed by cortical PV neurons, and has been shown to facilitate NMDA receptor function, would rescue ketamine-induced GBO abnormalities.

Tonic optogenetic excitation of BF PV neurons *in vivo* (n=6; 60 s constant 473 nm laser light at 5 mW) elevated spontaneous GBO and increased locomotor activity (n=4; 43%). Conversely, bilateral ArchT optogenetic inhibition of BF PV neurons in freely behaving mice (n=4; 2 min constant 532 nm laser light at 20 mW delivered at 2 min intervals) partially rescued the elevated spontaneous GBO activity elicited by acute ketamine (30 mg/kg). *In vitro*, using a slice model of GBO, CDPPB (10-20 μM; n=4) and CHPG (200 uM; n=8), positive modulators of mGluR5, while having no effect on the GBO alone, partially inhibited the acute potentiation of GBO by ketamine (100 μM).

Together, these findings suggest: 1) optogenetic manipulation of the activity of BF PV neurons is a non-pharmacological way to elicit Sz-like increases in spontaneous GBO and locomotor activity; 2) Inhibition of BF PV neurons or pharmacological facilitation of mGluR5 activity may be effective targets for modulating cortical E/I balance, and restoring normal GBO activity in Sz.

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Poster

507. Oscillations and Synchrony: Other II

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Topic: B.10. Network Interactions

Support: KAIST Institute

Title: Characterizing the large-scale network structure of spontaneous brain activity in mouse neocortex with voltage-sensitive dye imaging

Authors: *M. KANG[1,2], Y. LEE[1,2], B. GOHEL[3], Y. JEONG[1,2];
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Abstract: Spontaneous brain activity patterns in the neocortex are believed to represent underlying brain circuits and their functions. Indeed, recent neuroimaging studies have demonstrated that the functional connectivity and graph theoretical structure of spontaneous activity correlate with anatomical connections and behavioral performances. However, previous studies used imaging modalities that record hemodynamic signals of relatively low spatiotemporal resolution. Little is known about the large-scale network structure of neuronal signals with relatively superior spatiotemporal resolution.
To address this issue, we used voltage-sensitive dye imaging(VSDI) in mice, covering most neocortical regions of a single hemisphere. The signal was then used to characterize both static and dynamics of neural activation patterns. The effects of neuromodulators such as cholinergic agonist carbachol and norepinephrine were also studied.
Characterization of the static large-scale network connectivity pattern and its dynamics was based on connectivity matrix created with spatial independent component analysis-derived functional ROIs and pixel-by-pixel comparison. Graph theoretical analysis was then performed to identify hub regions and modules along with calculation of other parameters such as the global efficiency, clustering coefficient. Subsequently, dynamic changes of neural activation pattern were characterized. This included k-means clustering and quantification of ”local oscillation“ time-points to represent the number of less synchronized time-points.
We first identified default mode network-like hyperactivating regions that cover midline and temporal regions of the neocortex. The midline part of the regions was highly connected to the cortical hub regions along the parietal association areas. Further analysis revealed that norepinephrine and carbachol drives the functional connectivity to shift in distinct directions - norepinephrine increasing the cortex-wide activation spatiotemporally, leading to increased efficiency and decreased modularity while carbachol had the opposite effect. The “local”

oscillation duration was also higher in norepinephrine applied group and lower in carbachol applied group although other features was more robust to neuromodulator application.
Our results provide pixel-wise and large-scale analysis of graph theoretical parameters at a relatively superior spatiotemporal resolution with VSDI. We also see the effect of neuromodulators on the large-scale neuronal activity in distinct manners, preserving key features as such the hyperactivation pattern.

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Poster

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Topic: B.10. Network Interactions

Support: WT098352MA

R01 NS044133-14

Title: Physiological mechanisms underlying alpha frequency oscillations in the rat visual cortex.

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Abstract: Objectives: EEG alpha rhythms (8-12Hz) were the earliest brain rhythms discovered but remain the most mysterious in terms of mechanism. Functionally they play a fundamental role in cognitive processes such as selective attention and short-term memory. In the visual system, post-stimulus alpha oscillations are readily observed upon closing of the eyes or removal of visual stimulus. We therefore generated a post-stimulus model on activity in rat visual cortex *in vitro* to determine which physiological mechanisms are responsible for the generation of alpha rhythms.
Methods and Results: We isolated the V1 and V2 of the visual cortex from adult, male wistar rats in 450 μm thick slices. Extracellular field potential (LFP) recordings were taken from LIV of the V1 and gamma/beta2 activity was induced by application of 800nM Kainate (KA) to mimic sensory stimulation. This excitatory drive was then reduced by partial blockade of glutamate receptors. This manipulation alone reduced oscillation frequency to within the beta1 range and almost abolished LFP power. However, in the presence of cholinergic neuromodulation a near 4-fold increase in power was seen at alpha frequencies. This cholinergic effect could be mimicked entirely by blockade of I_h current: Control frequency 27.3 ± 0.6 Hz changed to 11. 2 ± 0.2Hz and

control power of 11.6 ± 1.7μV changed to 55.4 ± 8.2μV, n=51. The alpha rhythm dominated, as LFP, in layer IV of the V1. Intracellular studies showed 3 main types of LIV principal cell. Stellate cells and regular spiking, small pyramids spiked phase-locked to the control beta rhythm but not the alpha rhythm. Intrinsically bursting pyramidal cells spikes phase locked to both beta and alpha rhythms despite the presence of very little phasic synaptic inhibition. The dominance of this LIV bursting cell-associated alpha rhythm temporally uncoupled the majority of LIV principal cells from both LII/III and LV.
Conclusions: Alpha rhythms appear to dynamically uncouple the main thalamorecipient neurons in LIV V1 from other layers. We suggest this may represent the 'inhibition' of V1 by preventing ascending visual information from both passing on to higher order visual areas and also being influenced by top-down signal from these areas.

Disclosures: K. Hawkins: None. **A. Simon:** None. **R.D. Traub:** None. **M.A. Whittington:** None.

Poster

507. Oscillations and Synchrony: Other II

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Topic: B.10. Network Interactions

Support: Sloan Research Fellowship

Title: Data mining to generate novel hypotheses for the genetic underpinnings and functional roles of cortical oscillations

Authors: *P. SEBASTIAN[1], T. DONOGHUE[2], T. NOTO[2], S. HAXBY[3], B. VOYTEK[2]; [2]Cognitive Sci., [3]Mathematics, [1]UCSD, San Diego, CA

Abstract: Neural oscillations are ubiquitous across species, across cortical and subcortical brain regions, and may play a critical role in neural computation, function, disease states, and behavior. While it is known that oscillations are heterogeneous across the human brain, with specific oscillatory sub-bands manifesting in preferred regions, the degree of this heterogeneity and their physiological origins remains underexplored. Here, we align multiple spatial data into a common reference frame in order to explore the genetic origins and functional associations of neural oscillations. We use publicly available data including magnetoencephalography (MEG) data from the Open MEG Archive (OMEGA) and the Human Connectome Project (HCP), transcriptome-wide gene expression estimates from human brains using the Allen Human Brain Atlas (AHBA), and functional mapping data from Neurosynth. This allows us to make

comparisons between different methodologies and disciplines, to validate current theories of neural oscillations, and to generate novel hypotheses about their physiological origins and functional roles. Resting state MEG data (5-10 mins) were pre-processed and source-projected to a group template brain. Power spectra were calculated across all cortical locations and automated methods were used to estimate the 1/f noise exponent, and to identify the center frequencies and bandwidths of any existing oscillations, at each location. Group oscillation maps were calculated by creating a normalized score, encompassing the probability of an oscillation weighted by its normalized power, which we compute for a priori oscillatory bands: theta, alpha, beta, and low gamma. For each oscillatory topography, we compute a spatial correlation with functional term maps from Neurosynth, and gene expression maps from the AHBA. Spatial correlations between oscillatory maps and functional terms corroborate known associations, for example, theta correlates highly with 'episodic memory', alpha correlates highly with 'vision', and beta with 'motor'. We also find that oscillatory maps correlate with expression of many specific genes, including ion channel subtypes. These results show that disparate databases can be integrated in ways that support previous findings and suggest future hypotheses for the origin and function of neural oscillations.

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Poster

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Topic: B.10. Network Interactions

Support: FAPESP 2014/04568-4

Title: Gap junction channels in the synchronization of neuronal oscillations in a model of neuronal activation

Authors: ***E. R. KINJO**[1], M. S. A. FERRAZ[1], B. A. SANTOS[1], D. S. KOSTECKI[1], L. M. SILVA[1], A. C. VALLE[2], A. H. KIHARA[1];
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Abstract: The involvement of gap junction channels (GJC, composed of protein subunits called connexins-Cxs) in the synchronization of neuronal oscillations has been increasingly demonstrated, highlighting the importance of electrical synapses in shaping the neuronal

electrical activity. While most efforts have been dedicated to the understanding of chemical synapses, less is known regarding the plastic properties of electrical synapses. By using an *in vivo* model of neuronal activation induced by pilocarpine, our goal was to evaluate the effects of intrahippocampal administration of the GJC blocker carbenoxolone (CBX) in the hippocampal and cortical local field potentials (LFP). Male wistar rats were submitted to stereotaxic surgery for implantation of hippocampal cannula and cortical and hippocampal electrodes. After period of recovery, the animals were submitted to thirty minutes of recording of the basal activity. After that, animals were treated with methyl scopolamine (1 mg/kg; subcutaneous) followed by intraperitoneal pilocarpine (360 mg/kg) injection. Control animals received saline instead of pilocarpine. Thirty minutes after the establishment of status epilepticus, animals received intrahippocampal CBX (162 mM). The power spectrum density and spectrogram analysis revealed that the basal recordings from both cortical and hippocampal areas presented potentials that oscillated in the theta, beta and gamma frequencies, pattern that was not changed after methyl scopolamine administration. After pilocarpine injection, evident increase of the power of all the frequencies was observed, especially in the beta and gamma ranges, which were the same frequency ranges that presented large reduction of power after CBX administration, with smaller contribution than the observed during the basal period. After dissipation of the effects of CBX, ictal potentials were seen again, but the distribution of the frequencies was different from the detected before CBX administration, once the power of beta and gamma frequencies was significantly minor, suggesting that GJC has a fundamental participation in higher neuronal oscillations.

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Poster

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Topic: B.10. Network Interactions

Title: Spatio-temporal modeling of a macroscopic brain tissue from multi-cells level to single proteins using gaming technology

Authors: *U. NEVO, E. OPHIR, A. LIBERMAN, D. KARIO, M. MUSSEL;
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Abstract: We have created a generic platform for three dimensional, graphical, interactive simulations of macroscopic cerebral volume. Uniqueness of the software method is in: (a) its

capability to model the **spatial interactions of the neural network in 3D;** (b) modeling entities scaling from the level of cellular networks and down to single molecules and ionic fields (dynamics of concentrations of extracellular ions or of single proteins); (c) inclusion of chemical and biophysical interactions; (d) interactivity and user friendliness; The system features a graphical user interface to generate an experiment and displays an animated graphical representation of the experiment. The user may control the timeline of the experiment as well as intervene to affect the experiment itself (e.g. inject molecules). Two modes of operation are possible: (a) A “batch mode” is used to run multiple instances of the simulation with different parameters. The output is textual, including events and quantities from the experiment. It can easily be parsed, manipulated using "big data" tools, and graphed. (b) A real time (3D) animated view mimicking microscopy imaging of the cells, of membranal proteins that are visible as they diffuse and migrate on the surface and of action potentials. The "live" animation (not a movie, but a clip of simulation results!) allows to control the timeline of the experiment or the zoom and angle of the "microscope camera". The systems features oup to hundreds of neurons all operating in parallel, within a grid of 1 mm^3 tissue segment. Cells are 'decorated' by thousands of receptors and membrane proteins. Modeled biophysical and molecular mechanisms include: membrane excitability, release and diffusion of ions, apoptosis, release of neurotransmitters or other proteins, and more. Cellular actions are directed by state machine that defines signaling and response to environmental cues. Coding uses C++ and CUDA and the Unity3D game engine with its physics engine, scripting environment and GUI creator. We suggest the system for preliminary testing of hypotheses, optimization of experiments on neural systems (prior to "wet" experiments) and understanding of scenarios in which the exact spatial organization of the network is critical. Relevant pathological scenarios to be studied include the analysis of events such as **epileptic seizures, spreading depression or stroke** (with respect to the wave of secondary degeneration) or mechanisms of neurodegeneration. Our vision is that Cell Studio will become a ‘game-changer’ in neurobiology, and specifically in experimental biology, the same way simulations are used in engineering, physics and structural chemistry.

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Poster

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Support: Research supported by the Institue for Medical Engineering and Science, Massachusetts Institute of Technology

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Title: Thalamic generation of propofol phase amplitude coupling

Authors: *A. SOPLATA[1], J. SHERFEY[1], E. N. BROWN[4,5,7], P. PURDON[4,6], N. KOPELL[2,3]; [1]Grad. Program in Neurosci., [2]Dept. of Mathematics and Statistics, [3]Cognitive Rhythms Collaborative, Boston Univ., Boston, MA; [4]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA; [6]Dept. of Anesthesia, Critical Care and Pain Med., [5]Harvard Med. Sch., Boston, MA; [7]Dept. of Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hosp., Boston, MA

Abstract: Propofol, the most widely-used intravenous anesthetic, leads to both slow wave (0.1-1.5 Hz) and alpha (8-13 Hz) EEG oscillations at anesthetic doses. At these doses, propofol potentiates GABAa currents and antagonizes H-currents. At the deepest anesthetic level, there is phase-amplitude coupling (PAC) between the alpha and slow wave oscillations in which the alpha appears at the peak of the slow wave ("peak-max"). By contrast, at weaker doses corresponding to the time of loss or recovery of consciousness, the alpha appears in the trough of the slow oscillation ("trough-max") (Purdon et al., 2013). This change in PAC allows real-time determination of the depth of anesthesia (Purdon et al., 2013). To examine the mechanisms of this change in PAC, we simulated a thalamic system built from (Ching et al., 2010) and (Destexhe et al., 1996) consisting of thalamocortical and reticular nucleus cells with Hodgkin-Huxley dynamics and stochastic corticothalamic spiking input. We included propofol dose effects by increasing maximal conductance and decay time constant of GABAa-mediated inhibition by either 2X or 3X normal, and by decreasing maximal H-current conductance (gH). We then considered for each a pair of 2-D parameter spaces, varying gH and applied current corresponding to peak and trough phases of the cortical slow wave. The changes in the applied current allowed us to explore differences in voltage associated with changes along the slow wave and its effects on the thalamus' ability to oscillate at alpha frequency. Another difference between peak and trough states is that the former includes spiking cortical input. The value of gH was shown to be important in the creation of the alpha oscillations at either peak-max or trough-max. We found regions of alpha frequency oscillation and quiescence within this parameter space corresponding to peak-max when GABAa is 3X normal and trough-max when GABAa is 2X normal. These results suggest that the thalamus has the intrinsic mechanisms to produce the observed switch in phase-amplitude coupling.

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Poster

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Title: Arc/Arg3.1 and c-fos changes in response to *In vitro* models of wake and sleep-related cortical dynamics.

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Abstract: Objectives: Immediate early genes (IEGs) are rapidly transcribed after certain cellular cues. In studying the cortical environment during sleep and wakefulness two IEG are of particular interest: c-fos and Arc. C-fos signals an increase in neuronal activity whereas ARC has a complex association with synaptic plasticity involving both AMPA receptor internalisation and synaptic stabilisation depending on dynamics and magnitude of activation. Here we use markers for c-fos and Arc to differentiate regional and laminar cortical changes in network dynamics associated with non-REM sleep and cortical activation.
Methods & Results: Acute neocortical slices from adult rats containing primary sensory (S1 or Au1) and association (Par2) areas were maintained in vitro. Activation-associated (wake-state) rhythms (gamma (30-80 Hz) and beta (15-25 Hz)) were induced by bath application of kainate (400nM). Non-REM sleep-associated rhythms (delta (1-4 Hz)) were generated by bath application of 10μM SCH23390 and 4μM Carbachol. After 1hr of stable rhythms slices were fixed and immunohistochemistry performed for NeuN, GAD67, and c-fos and Arc.
Comparing delta to gamma/beta oscillations, c-fos levels were reduced by 19% in association cortex. This decrease was similar in all layers. In contrast, primary sensory areas showed only a 7% reduction in c-fos levels. Arc also showed a larger change (albeit in the opposite direction) in association cortex (55% increase in delta) than primary sensory cortex (36% increase). In addition, the laminar distribution of these ARC changes was different in each area: The majority of the ARC signal increase in primary sensory cortex was seen in supragranular layers, whereas in association cortex this was in layer 5 and the main apical dendrites ascending from layer 5 pyramidal cells.
Conclusions: A far greater change in IEG expression was seen in association cortex compared to primary sensory areas when comparing wake-related rhythms to non-REM sleep-related rhythms. The largest change observed (ARC) suggested an overt alteration in the environment supporting synaptic plasticity in output neurons from higher-order cortical regions during deep sleep.

Disclosures: **I.J. Hartnell:** None. **A. Simon:** None. **S. Chawla:** None. **M.A. Whittington:** None.

Poster

507. Oscillations and Synchrony: Other II

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Title: Beta oscillations in neocortex: A multiscale modeling study

Authors: ***S. A. NEYMOTIN**[1,2,3], S. DURA-BERNAL[2,4], B. A. SUTER[5], P. LAKATOS[6,7], G. M. G. SHEPHERD[5], W. W. LYTTON[2,8];
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Abstract: Beta oscillations (15-30 Hz) are prevalent in the firing patterns of neocortical neurons and the microcircuits they are embedded in. Beta oscillations are hypothesized to enable effective communication between neurons, setting fluctuating windows of excitability when neurons are most likely to respond to or send information. Beta oscillations are expected to emerge from interactions across multiple spatial scales, arising from a diverse set of biophysical mechanisms. Key contributors to Beta oscillations are the Layer 5 (L5) pyramidal neurons, with their large dendrites which span multiple cortical layers. To explore the origins of neocortical Beta oscillations, we developed a 6-layer 1715 compartmental cell multiscale model of neocortex with multiple classes of excitatory (E) and inhibitory (I) neurons wired based on mouse neocortex circuit mapping experiments. We optimized models of L5 pyramidal cells using whole-cell somatic voltage recordings. Neurons contained membrane calcium, sodium, potassium, and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. In L5 pyramidal cells, increasing HCN channel density in apical dendrites with distance from soma promoted synaptic resonance in the Beta frequency range, with increasing resonant frequency

with distance from soma. The network model displayed coherent Beta oscillations produced through interactions between E and I neurons. We applied independent random variations in ion channel densities to cells in the network model to identify parameter sets that contributed to Beta oscillations. These variations produced simulations with distinct physiological (phys) and pathological (path) hyperexcitable states, each with different expressions of Beta oscillations, neuronal synchrony, and firing patterns. The simulation categories demonstrated degeneracy: there were many parameter combinations that produced each simulation type with weak intra-class similarity of parameter vectors (phys/phys r=0.06; path/path r=0.07; n=65 for each class) and weak inter-class dis-similarity (path/phys r=-0.05). Some of the model predictions were verified through analyzing Beta oscillations in laminar neocortex recordings of nonhuman primates. Our results suggest the neocortex contains a robust set of mechanisms that contribute to Beta oscillations at multiple spatial scales.

Disclosures: **S.A. Neymotin:** None. **S. Dura-Bernal:** None. **B.A. Suter:** None. **P. Lakatos:** None. **G.M.G. Shepherd:** None. **W.W. Lytton:** None.

Poster

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Topic: B.10. Network Interactions

Support: NSF Grant EEC-1028725

NIH Grant NS065186

Title: Reliability of electrophysiological connectivity in the resting state.

Authors: *K. CASIMO, T. MADHYASTHA, J. G. OJEMANN, K. E. WEAVER;
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Abstract: A growing body of literature exists on variability in resting state functional connectivity. Studies of fluctuations in BOLD signal measured using fMRI have indicated that connectivity patterns are not static, but rather vary across time scales and between sessions. However, fMRI is limited to indirect measurement of brain activity driven by delayed-response blood flow. Resting state connectivity dynamics have received substantially less investigation using electrophysiology.
In this study, we used electrocorticography (ECoG) with video monitoring to identify multiple eight minute resting state sessions from epileptic individuals undergoing seizure monitoring. We

assessed connectivity using a range of interaction measures including linear regression, phase locking value, and phase slope index. To assess change between sessions, we calculated interclass correlations on mean connectivity values and used a mixed model regression approach. We sought to investigate reliability in connectivity across highly sampled brain regions at rest; thus, connectivity changes were assessed among all Brodmann area (BA) regions with sufficient data rather than known resting state networks. Electrode BA labels were identified using the MNI atlas.
We found connectivity changes spanned a wide range of cortical regions. We found regional variability in connectivity between spatially distant areas including prefrontal, superior parietal, and lateral and inferior temporal regions. We observed both increases and decreases in connectivity across canonical frequencies from delta (0-4Hz) through high gamma (70-200Hz) as well as changes in the 0.1-1Hz amplitude modulations of high gamma, which has been previously demonstrated to closely correspond to, and potentially give rise to, low-frequency BOLD signal. Variations were observed across the multiple connectivity measures.
The resting state connectivity trends we observe with electrophysiological techniques are consistent with the variability previously attributed to the resting state by studies using BOLD techniques. Here, we add the novel dimensions of direct measurement of neural activity using ECoG, a broad frequency range from 0-200Hz, and the use of multiple complementary connectivity measures. We estimated the natural variability in connectivity across a variety of electrophysiological metrics of the resting state, with the potential to illuminate critical cognitive functions over time, including memory and skill consolidation. This method will facilitate study of changes in resting state connectivity to interventions, such as learning of a skill, which are above and beyond natural variation.

Disclosures: **K. Casimo:** None. **T. Madhyastha:** None. **J.G. Ojemann:** None. **K.E. Weaver:** None.

Poster

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ICM P10-001-F

The Guillermo Puelma Foundation

Title: Histamine H1 receptor antagonist pyrilamine induces gamma oscillations in *In vivo* rat CA1 hippocampus

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Abstract: Histamine is known to be involved in the regulation of many physiological process such as sleep/wake cycles, motivation, arousal and, recently in learning and memory. In freely moving animals, histamine H1 receptor (H1) antagonists has been shown to increase the abundance of sharp-wave ripples in the hippocampal CA1 region, widely associated with memory consolidation. Histamine acting intracellularly, in a similar fashion as serotonin and acetylcholine, has been shown to increase neuronal excitability by enhancing I_h and blocking the Ca^{2+}-sensitive K^+ channel responsible for the slow-afterhyperpolarization.
It has been thought that gamma oscillations (30-120 Hz), and their corresponding neuronal activity, underlie a variety of cognitive functions. *In vitro* studies have suggested that the generation of gamma oscillatory patterns is better explained by a pyramidal-interneuron network gamma (PING) model, however, the mechanisms by which this oscillation is generated *in vivo* is still not clear. In *in vitro* preparations, application of the cholinergic agonist charbacol induced gamma oscillations in CA3. High concentrations of kainate can also elicit gamma oscillation in the CA1 region. Given the involvement of histamine in hippocampus-dependent cognitive function, we investigated the effect of direct injection of the H1 antagonist pyrilamine on the oscillatory patterns of the CA1 region. Sprague-Dawley rats were implanted with an infusion cannula into CA3 together with an 8-tetrode hyperdrive aiming to record the oscillatory activity of the CA1 layer. The rat was habituated to sleep into a flowerpot while pyrilamine was slowly infused into the CA3 layer of the hippocampus. At the same time, LFP and single unit recordings from CA1 layer were obtained. Our study shows that acute perfusion of the H1 antagonist pyrilamine into the CA3 layer induced a high- and low-frequency gamma oscillation in the CA1 layer of the hippocampus. This result presents a novel effect of the H1 receptor antagonist pyrilamine in the oscillatory patterns of hippocampal CA1 region.

Disclosures: C.A. Villalobos: None. **P. Maldonado:** None. **J. Valdes:** None.

Poster

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International Graduate School of Neuroscience, Ruhr University Bochum

Wilhelm and Guenter Esser Foundation

Title: Spiking regimes in model networks of hippocampal persistent firing neurons

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Abstract: Rapid persistent firing is thought to be the neural correlate of short-term memory maintenance (Fuster 1971), in which transient stimuli must be preserved during long delay periods outlasting them. Therefore, the brain structures commonly involved in working memory tasks, including the hippocampus, must possess the necessary substrates for the generation and maintenance of elevated persistent activity. Various synaptic mechanisms underlying mnemonic persistent firing have been suggested by neuroscientists. The common view is that networks with specific topologies, such as recurrent excitatory connections, depend on strong local excitation for the generation of long-lasting neuronal activity and rhythmic oscillations (Wang 2001). However, it has been shown that memory can also be encoded in brain regions which do not display such topologies, such as the CA1 area of the hippocampus (Amaral 1993). These regions display similar activation patterns, and must therefore rely on mechanisms which are independent of topology and connectivity, such as intrinsic cellular properties. Among these are the ionic currents mediated by calcium-activated non-specific (CAN) ion channels, which seem to be a valid candidate for rhythm generators in hippocampal networks.
Our work investigates the different patterns of activity displayed by model networks of hippocampal CAN-equipped persistent firing neurons. The neuron model is based on the Hodgkin-Huxley formalism for increased biological plausibility. We show that synchronized patterns elicited by a transient (250 ms) stimulus can be maintained solely by CAN currents, without the need for strong recurrent connections. In addition, such a CAN-network exhibits three main types of firing regimes depending on the value of the connection weights - a slow regime with firing rates $6\ \text{Hz} < f < 18\ \text{Hz}$, a bursting regime, and a fast gamma regime with firing rates $f > 40\ \text{Hz}$. These results are in accordance with in vitro recordings of hippocampal slice preparations (Knauer et al. 2013), and provide a possible explanation for the generation and maintenance of memory-related oscillatory activity in different frequency bands, within the hippocampus.

Disclosures: **F. Giovannini:** None. **B. Knauer:** None. **M. Yoshida:** None. **L. Buhry:** None.

Poster

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Topic: B.10. Network Interactions

Title: Lactate cannot fully sustain gamma oscillations but sharp wave-ripple activity *In vitro*

Authors: *J. SCHNEIDER, J.-O. HOLLNAGEL, T. CESETTI, A. LEWEN, O. KANN;
Inst. of Physiol. and Pathophysiology, Heidelberg, Germany

Abstract: The sensitivity of higher brain functions to hypoxia and hypoglycemia implies that network activities, which underlie complex neuronal information processing, rely on the sufficient availability of energy substrates. While it is established that glucose can fuel neural network activity, it has been proposed by the astrocyte-neuron lactate shuttle hypothesis that lactate is the preferred energy substrate for neurons while glucose is primarily taken up by astrocytes. In this study we used local field potential recordings in organotypic hippocampal slice cultures and acute slice preparations from the rat to test if lactate can fuel gamma-oscillations and sharp wave-ripples. These network activities naturally occur in the hippocampus in vivo and are associated with memory formation and consolidation, respectively. Gamma-oscillations were induced in slice cultures that were supplied with 2 to 20 mM lactate and subsequently with 10 mM glucose containing ACSF. We found that the number of slice cultures that expressed gamma-oscillations and the oscillation power was reduced in the presence of lactate compared to glucose. In acute slices gamma-oscillations were abolished in the presence of 10 mM lactate. When slices were supplied with 20 mM lactate, gamma-oscillations were smaller compared to 10 mM glucose and interrupted by epileptiform activity. On the contrary, sharp wave-ripples evoked in acute slices had similar characteristics (amplitude, frequency) in the presence of both glucose (10 mM) and lactate (20 mM). Additionally, we estimated oxygen consumption rates in acute slices from oxygen recordings with Clark-type electrodes. We found that oxygen consumption rates were lower during sharp wave-ripple activity compared to gamma oscillations. Summarizing, we found that lactate can fully sustain sharp wave-ripples, but not gamma oscillations. Since oxygen consumption rates during sharp wave-ripples were lower than during gamma oscillations, this indicates that the sharp wave-ripple network activity state express a lower energy expenditure. Together, these findings suggest that the metabolic rate of a specific network activity state might determine if it can be exclusively fueled by lactate or not.

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Poster

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Title: Parvalbumin-positive interneurons regulate spatio-temporal network dynamics for population coding in mouse primary visual cortex

Authors: *M. AGETSUMA[1,2,3], J. HAMM[3], I. SATO[4], R. YUSTE[3];
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Abstract: For efficient information processing in the brain, neural circuit dynamics must be spatially and temporally regulated with great precision. Although parvalbumin-positive (PV) interneurons are known to control network synchrony, how their activity contributes to spatio-temporal activity patterns remains unclear. We investigated this using *in vivo* two-photon Ca^{2+} imaging from populations of neurons in mouse visual cortex with simultaneous optogenetic inactivation of PV cells. As expected, network synchrony in visual cortex was locally sustained by PV interneurons. But interestingly, the reliability of multineuronal activity during visual stimulation was specifically disrupted by PV-cell suppression. Machine-learning based decoding confirmed the importance of PV cells in population coding, and further correlation analyses suggested the link between distance-dependent regulation of network synchrony by PV cells and the underlying computation. Our study indicates that interneurons can control the spatio-temporal dynamics of cortical information, and therefore are critical for building a population code.

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Poster

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Title: Chemokine effects on cerebellar oscillations *In vitro*.

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Abstract: Objectives: Besides their well-established role in the immune system chemokines have been suggested to play a role in the central nervous system (CNS). Recent studies clearly demonstrate that these small cell signalling molecules and their receptors are constitutively expressed by glial cells and neurons, where they are involved in intercellular communication. They also have a role in neuroinflammation, neurodegenerative diseases and depression. We have investigated the effects of two different chemokines (recombinant murine KC/CXCL1(KC) and recombinant murine rantes/CCL5(rantes)) on cerebellar gamma oscillations in Chemokine (C-C motif) Receptor 5 (CCR5)- and Atypical Chemokine Receptor1 (ACKR1)- deficient mice and on wild type (WT) animals.
Methods and Results: Using 450 μm coronal cerebellar slices from adult male mice, oscillations were induced either through G-protein coupled metabotropic glutamate receptors (mGluR) by bath application of 10 μM (S)-3,5-Dihydroxyphenylglycine (DHPG) or through nicotinic acetylcholine receptors (nAchR) by adding 10 μM nicotine (NIC). Both methods induced a robust and persistent high gamma frequency oscillation (60-80Hz) with an element of very fast oscillation (VFO, >100 Hz). Local field potentials (LFPs) were recorded from crus1 and crus2 areas associated with expectancy and non-motor shifts in attention. In WT animals 100ng/ml KC decreased the power of both DHPG- and NIC induced oscillations by 30% and 80%, respectively. 10 ng/ml rantes did not change the DHPG oscillation but decreased the NIC induced oscillation by 50%, leaving the frequency components unaffected. In tissue from the ACKR1 mutant mouse DHPG and NIC failed to evoke cerebellar oscillations of a similar power to control; evoked gamma oscillations were ~80% smaller and occurred in the absence of VFO. In these experiments KC also failed to modulate gamma/VFO rhythms in either case. However, rantes selectively doubled the power of the NIC-evoked oscillation in the ACKR1 mutant; introducing high frequency gamma and VFO. Since the ACKR1 and CCR5 receptors can also hetero-oligomerize and rantes is a ligand for both receptors, we investigated the effects of rantes on CCR5 deficient animals too.

Conclusions: Our results show that chemokines might be able to significantly alter oscillatory dynamics in a manner where selectivity is dependent on the presence of the ACKR1 protein.

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Poster

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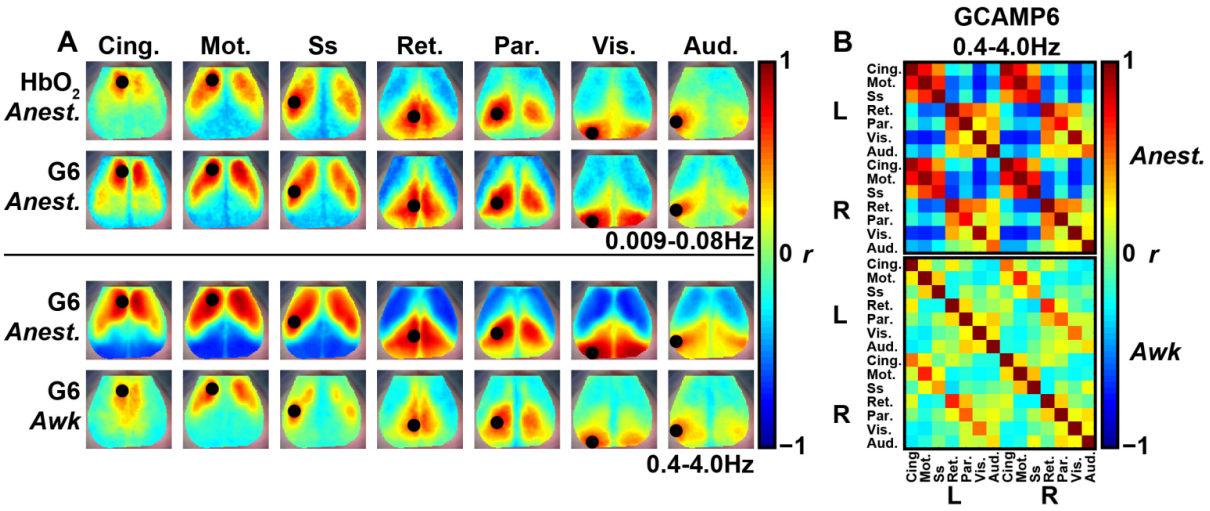
Title: Functional connectivity structure of cortical calcium dynamics in anesthetized and awake mice

Authors: ***P. WRIGHT**, A. Q. BAUER, G. A. BAXTER, M. D. REISMAN, A. BICE, J. P. CULVER;
Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Hemodynamic-based markers of cortical activity (e.g. fMRI, optical intrinsic signal imaging), driven by electrical and metabolic activity through neurovascular coupling, are an indirect and slow report of brain function and are limited in their utility to deduce underlying brain network dynamics. Here we extend functional connectivity (FC) analysis, a method for mapping functional relationships using spontaneous brain activity, from hemodynamic to Ca^{2+}-dynamic imaging. Transgenic mice (n=7) expressing a fluorescent calcium indicator (GCAMP6) driven by the *Thy1* promoter in cortical glutamatergic neurons were imaged transcranially in both ketamine-anesthetized and awake states. Sequential LED illumination (λ=470, 530, 590, 625nm) enabled concurrent imaging of both GCAMP6 fluorescence emission (corrected for hemoglobin absorption) and hemodynamic activity. Somatosensory responses were evoked using a 0.5mA electrical hindpaw block paradigm. FC patterns were generated for low (0.009-0.08Hz) and high (0.4-4Hz) frequency bands. Following paw stimulation, GCAMP6 provided a response

time course sensitive to individual high frequency (2Hz) pulse presentations and preceded the stereotypical hemodynamic response function by ~0.65s (data not shown). Homotopic HBO2 and GCAMP6 FC maps have similar topographies at low frequencies (Fig 1A). At higher frequencies, GCAMP6 is sensitive to delta band (0-4Hz) activity associated with slow-wave sleep which provides a striking effect on the correlation structure of the FC maps from anesthetized mice that is diminished upon wakefulness (Fig 1A, & Fig 1B for a pairwise matrix of correlation values across networks). In summary, functional neuroimaging of Ca^{2+} dynamics in mice provides evidence that spatiotemporal coherence in cortical activity is not exclusive to hemodynamics. Concurrent Ca^{2+} and hemodynamic-based imaging will enable the dissociation of changes in ionic networks, hemodynamic networks, and neurovascular coupling and provide a framework for subsequent studies of neurological disease, such as stroke.



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Poster

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AA005965

Title: Acute alcohol attenuates regional bilateral connectivity in the rat brain

Authors: *N. M. ZAHR[1], D. KWON[3], E. T. PETERSON[3], E. V. SULLIVAN[2], A. PFEFFERBAUM[3];
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Abstract: Acute, binge, and chronic alcohol exposure have distinct effects on the brain. Although the effects of acute ethanol (EtOH) exposure on various magnetic resonance imaging (MRI) metrics have been evaluated in humans, systematic evaluations of the effects of acute, binge, and chronic EtOH administration on the brain have not been conducted in animal models. This resting-state functional MRI (rsfMRI) study was conducted in 12 wild-type Wistar rats (425.8±25.5g) at 4 time points: baseline (scan 1); following acute intragastric administration of 4g/kg EtOH (resulting in blood alcohol levels (BALs) of ~300mg/dL) or saline (scans 2 and 3) with random assignment; and following 2g/kg EtOH (scan 4, with BALs ~150mg/dL). Animals, anesthetized with dexmedetomidine (0.1mg/kg/h s.c.), were scanned on a 7T Bruker imaging system. rsfMRI analysis utilized our in-house rat brain atlas, including 68 labels comprised of 24 bilateral and 20 midline regions of interest (ROI), constructed using the Paxinos/Watson atlas and wire frame images (http://www.nitrc.org/projects/jip). fMRI image processing included motion correction, registering and warping structural to fMRI space, registering to template space, warping fMRI to template space, and cropping. Subsequent processing was conducted in atlas space. Pre-processing used AFNI with high and low pass filtering and smoothing with a Gaussian kernel. Processed fMRI data was mapped into atlas space by registering mean fMRI images to our rat atlas. For connectivity analysis, correlation map computation involved mean fMRI signal calculation for each ROI and computing correlations between mean fMRI signals in each ROI. Correlation maps showed significant bilateral regional connectivity at baseline, which was attenuated more by 4g/kg EtOH than by 2g/kg EtOH. The regions showing the greatest signal loss - visual and auditory cortex, temporal association cortex, and inferior colliculs - are among the regions with the highest cerebral blood flow and deoxyglucose uptake. These results suggest that acute EtOH administration initially disrupts activity in brain regions that typically consume the greatest energy.

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Poster

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Support: Universal 2014, CNPq

Title: Salicylate induces type 2 theta oscillation in the hippocampus

Authors: *R. N. LEAO[1], R. FRANZON[1], S. MIKULOVIC[2];
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Abstract: Salicylate in high doses is cause of tinnitus in humans and it is often used to produce tinnitus-like perception in animal models of the condition. Here we assess whether salicylate induces anxiety-like electrophysiological and behavioural signs. Using multielectrode probes, we recorded local field potential (LFP) in the dorsal and ventral hippocampus in an open field 60-min after salicylate (300mg/kg) injection. We found that animals treated with salicylate moved dramatically less than saline treated animals. Consequently, dorsal hippocampus theta (8-9Hz, associated with movement) in these animals was significantly lower. However, salicylate-treated animals showed a strong 4-7Hz oscillation in the ventral hippocampus (with smaller peaks in dorsal hippocampus electrodes). These oscillations were abolished by atropine application, suggesting that this rhythm is a theta type 2 oscillation (atropine sensitive). Granger causality analysis demonstrated that the dorsal hippocampus Granger-caused 8-9Hz theta in the ventral hippocampus while the ventral hippocampus Granger-caused type-2 theta in the dorsal hippocampus in salicylate-treated animals. Theta type 2 is elusive to be elicited in rodents and have been associated to anxiety-like behaviours. Here we show that salicylate application can consistently generate type 2 theta in the ventral hippocampus. Future studies will show whether ventral hippocampus type 2 theta is caused by salicylate itself or by tinnitus perception.

Disclosures: **R.N. Leao:** None. **R. Franzon:** None. **S. Mikulovic:** None.

Poster

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Title: Stimulus specificity and task condition dependence of field potential oscillations in the solitary nucleus

Authors: *A. DENMAN-BRICE, P. M. DI LORENZO;
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Abstract: Synchronous neural activity has been found between several cortical and subcortical regions of the rodent taste system. The role of oscillatory activity in the nucleus of the solitary tract, however, has not been investigated. The solitary nucleus, which is located in the brainstem and receives descending projections from numerous cortical and subcortical structures, integrates information across many sensory modalities, including olfactory and viscerosensory, and contains neurons that convey information about stimulus identity through temporally precise codes. We analyzed recordings from rats with chronically implanted 8-channel microwire electrodes in the solitary nucleus. Spiking activity of single units and field potential signals were recorded as the rats freely licked taste solutions from a computer controlled lickspout. In some recording sessions, taste stimuli were presented in random order, and in other sessions a subset of the same stimuli were used in a go / no go task, which required subjects to attend to specific stimuli in order to obtain rewards and avoid punishment. Analysis of field potential signals revealed the presence of a robust oscillation in the theta range, which was phase locked to licking activity but sometimes occurred in the absence of licking behavior and sometimes absent during licking behavior. Power of this oscillation following stimulus presentation was reliably modulated by stimulus identity, but the pattern of stimulus modulation differed across subjects and between the random stimulus presentation paradigm and the go / no go paradigm. Analysis of single units revealed that the firing of a subset of neurons previously identified as lick-related was better correlated with phase of the local field oscillation than with licking. Additionally, some neurons in the NTS synchronized with the local oscillation more strongly following presentation of some stimuli and others. Another subset of neurons was identified which consistently fired during different phases of the local field oscillation depending on which stimulus was presented. These results suggest that field potential oscillations in the solitary nucleus may reflect processing of sensory information and/or the influence of top-down control.

Disclosures: A. Denman-Brice: None. **P.M. Di Lorenzo:** None.

Poster

507. Oscillations and Synchrony: Other II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 507.26/P11

Topic: B.10. Network Interactions

Support: DARPA N66001-14-C-4066

CIHR MOP-115178

Title: Transcranial direct current stimulation facilitates associative learning and alters functional connectivity in the non-human primate brain

Authors: M. R. KRAUSE[1], T. P. ZANOS[2], B. CSORBA[1], M. PHILLIPS[3], P. K. PILLY[3], *C. C. PACK[1];
[1]Neurol & Neurosurg, McGill Univ., Montreal, QC, Canada; [2]Feinstein Med. Inst., Manhasset, NY; [3]Hughes Res. Labs., Malibu, CA

Abstract: Transcranial direct current stimulation (tDCS) is a non-invasive technique for modulating brain activity. Given its safety and low cost, interest in tDCS has grown rapidly in recent years, and there is now some evidence that tDCS has therapeutic value for a range of neurological and psychiatric disorders, including stroke, depression, addiction, and schizophrenia. Other work claims that tDCS affects healthy subjects' perceptual and cognitive performance, as well as their creativity. At the same time, very little is known about how tDCS influences activity in the human brain, and there is some debate about whether it has any effect at all. Here, we examine the influence of tDCS on multi-site neural activity in awake, behaving non-human primates, which provide the best available animal model of the human brain. Monkeys performed an oculomotor foraging task, which required them to learn the association between a natural visual image and a specific location within that image. Animals received liquid rewards in return for executing saccades to this location, and they were required to learn different locations for different images. This task allowed us to separate potential effect of tDCS on learning from those on visual processing, motor performance, and overall attention and arousal. During performance of this task, we recorded simultaneously from multiple sites within prefrontal cortex (PFC) and inferotemporal cortex (ITC), using chronically implanted multi-electrode arrays. In alternating blocks of trials we applied "sham" stimulation, which simulated tDCS while delivering no current to the brain.
We found that, compared to sham stimulation, anodal tDCS applied to prefrontal cortex (PFC) accelerated the learning of new associations, and that this increased plasticity was accompanied by altered functional connectivity between PFC and ITC. Specifically, tDCS increased oscillatory coherence between the prefrontal and visual cortices in the theta band (6 - 10 Hz) of the local field potentials. This increased coherence was specific to the period within each trial when the association was being learned or encoded; tDCS had no effect on coherence during the initial period following the presentation of each image. Moreover, there was no consistent effect of tDCS on single-neuron firing rates or stimulus selectivity; effects on the local field potentials at individual sites were broadband and similar for different time periods within the trial. These results show that tDCS is capable of modulating learning and its neural correlates in the primate brain.

Disclosures: **M.R. Krause:** None. **T.P. Zanos:** None. **B. Csorba:** None. **M. Phillips:** None. **P.K. Pilly:** None. **C.C. Pack:** None.

Poster

507. Oscillations and Synchrony: Other II

Location: Halls B-H

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Program#/Poster#: 507.27/P12

Topic: B.10. Network Interactions

Support: NSF Grant 1056105

Title: Different isoflurane levels have distinct effects on cross-hemisphere functional connectivity during unconsciousness

Authors: *A. SZYMANSKA[1,2], M. ALCALA ALVAREZ[2], A. AFSHEEN BAZRAFKAN[3], M. FARAHABADI[3], Y. AKBARI[4], Z. NENADIC[2];
[1]BME, UCI, Irvine, CA; [2]Dept. of Biomed. Engin., [4]Dept. of Neurol., [3]Univ. of California Irvine, Irvine, CA

Abstract: Background: It is relatively well understood that brain states vary in awake and anesthetized subjects. The effects of anesthesia include, but are not limited to, the suppression of nonsynchronous responses, as well as general depression of neurological signals. However, few studies have investigated the effects of varying levels of anesthesia on spontaneous (baseline) neurological activity, as well as synchrony across the two brain hemispheres. In order to determine the effects of varying levels of anesthesia on cross-hemisphere spontaneous neurological activity, we have collected cross-hemisphere electroencephalogram (EEG) and multi-unit activity (MUA) on anesthetized rats. Methods: Rats were anesthetized using isoflurane (2.0%) and implanted with two EEG screw electrodes in the left hemisphere. A 2 mm burr hole was then drilled through the skull, and a 7-channel microelectrode was lowered into layer 2-3 of the cortex of the right hemisphere. Spontaneous EEG activity on the left hemisphere was then recorded simultaneously with spontaneous MUA activity from the right hemisphere. The isoflurane level was then increased to 2.5%, and recording was repeated after the neurological activity had stabilized (about 5 min). This was then repeated at 3.0% isoflurane. Results: As expected from previous studies, the overall EEG neurological activity decreased under anesthesia. This was also true for MUA. The activity continued to decrease as anesthesia levels were increased. Interestingly, the correlation between the right hemisphere EEG and left hemisphere spiking frequency increased as anesthesia levels increased. Furthermore, the maximum correlation shifted from the Theta band to the Alpha band with increasing anesthesia. We also calculated the spike-triggered average (STA) at each anesthesia level, by averaging over 150ms of EEG centered on individual recorded APs. Although less overall activity was present as anesthesia levels increased, the STA magnitude significantly increased. Conclusion: Cross-hemisphere interactions were affected by the level of anesthesia administered. As anesthesia levels were increased the correlation between the EEG and MUA increased, and STAs

significantly increased in magnitude. This may imply that higher anesthesia levels selectively inhibit background or asynchronous activity, causing the low level of activity present to be higher in magnitude and more correlated across the two hemispheres.

Disclosures: A. Szymanska: A. Employment/Salary (full or part-time): University of California, Irvine. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; NSF (1056105). **M. Alcala Alvarez:** A. Employment/Salary (full or part-time): University of California Irvine. **A. Afsheen Bazrafkan:** A. Employment/Salary (full or part-time): University of California Irvine. **M. Farahabadi:** A. Employment/Salary (full or part-time): University of California Irvine. **Y. Akbari:** A. Employment/Salary (full or part-time): University of California Irvine. **Z. Nenadic:** A. Employment/Salary (full or part-time): University of California Irvine. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; NSF (1056105).

Poster

507. Oscillations and Synchrony: Other II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 507.28/Q1

Topic: B.10. Network Interactions

Support: NIH Grant MH103592

NIH Grant MH103204

Title: Frequency bands are an organizational force of intrinsic brain networks

Authors: S. MOWLAEI[1], A. SINGH[2], *A. S. GHUMAN[1];
[1]Neurolog. Surgery, Univ. of Pittsburgh, Pittsburgh, PA; [2]Machine Learning, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Studies have increasingly demonstrated the importance of oscillatory dynamics in neural coding and interregional communication. While it has become clear that frequency-specific activity is a critical feature of brain activity, whether brain networks organize by "frequency band" or whether the spectrum does not enforce functional brain network organization. Specifically, here we ask if there is a functional parcellation of the frequency spectrum or not in resting-state magnetoencephalography (MEG) data based on large-scale brain

network connectivity patterns. To answer this question, we used network analyses and unsupervised and supervised pattern recognition algorithms in conjunction with whole brain MEG resting-state connectivity measures. Specifically, we recorded 5 minutes of eyes open, fixated resting-state MEG data from 18 healthy subjects. After artifact removal, in each subject, for each frequency from .5-50 Hz with 0.5 Hz steps, we calculated the source-localized, all-to-all connectivity matrix based on phase locking values between each pair of points on the cortex (5124x5124 connectivity matrix). Using matrix and network similarity measures, we then assessed the spatial similarity of these full connectivity matrices between each pair of frequencies. Unsupervised and supervised analyses were used to assess whether there was consistent groupings of frequencies across subjects. The results of this analysis show that there are between 5 and 6 data-driven frequency bands that organize intrinsic brain networks. These bands roughly correspond to classic frequency bands: .5-2.5 Hz (~delta), 3-9 Hz (~theta), 9.5-13 Hz (~alpha), 13.5-21 Hz (~low beta), 21.5-32.5 Hz (~high beta), and 33+ Hz (~gamma). The bands also show very high consistency across subjects allowing one to classify each frequency to the correct band at the single subject level with 90+% accuracy. The results also show that there is a substantial spatial overlap between the spatial topography of the theta and low beta bands (with 5 bands, these two frequency ranges are grouped together, with 6 they separate). Furthermore, the results show that the low and high beta bands are substantially distinct and therefore should not be grouped together. In addition, we used network analyses to determine the characteristic brain networks that correspond to each frequency band. Taken together, these results suggest that synchronized activity grouped by frequency bands is a strong and consistent organizing force of large-scale, intrinsic brain networks.

Disclosures: **S. Mowlaei:** None. **A. Singh:** None. **A.S. Ghuman:** None.

Poster

507. Oscillations and Synchrony: Other II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 507.29/Q2

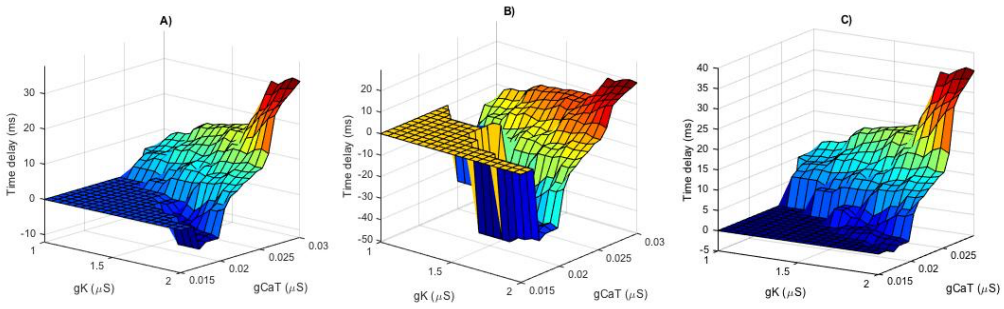
Topic: B.10. Network Interactions

Title: Modelling pyloric dilator neurons in the pyloric network

Authors: ***F. DOS SANTOS BRANDAO**, P. ANDRAS;
Keele Univ., Newcastle, United Kingdom

Abstract: Recent studies of the neurons in the Tritonia pedal ganglia and the spherical neurons in the electrosensory lobe of the electric fish have suggested that the same neuron can play different functional roles in the neural circuits to which it belongs. We study the pyloric rhythm

network of the crustacean stomatogastric ganglion (STG).
Most commonly the STG network models use single model neuron for the same kind of neurons, assuming the same parameters and implying their synchronous firing. However, simultaneous recordings of pyloric dilator neurons (PD) show differences between the timings of spikes. We built a pyloric network with nine neurons (AB - anterior burster, LP - lateral pyloric, two PD and five PY - pyloric neurons) using two-compartment (soma-dendrite) models for each neuron. The parameters of the model neurons are from published papers. We analysed the behaviour of the two model PD neurons. We varied the conductance parameters (g_K - delayed rectifier potassium and g_{CaT} - transient calcium) of one of the PD within functional ranges, while the other had fixed parameters.
Our results show that the PD neuron with higher g_{CaT} and g_K leads the spiking in the PD phase of the pyloric rhythm and have greater or equal number of spikes compared to the follower PD neuron. The leading role is determined primarily by the calcium conductance. The temporal delay between the corresponding spikes of the two PD neurons gets shorter if the potassium conductance of the follower is larger - see figure 1.
Figure 1 - Time distances of the first spike as a function of the g_K and g_{CaT} conductances of the neuron with variable conductances. A) From literature, B) lower conductances and C) higher conductances.
The simulation results show that some combinations of potassium and calcium conductances lead to abnormal spiking behaviour in the model neurons. Based on the activity and spiking patterns we defined two abnormal behaviours that were excluded from further analysis.
Our work aims to establish a robust and accessible computational and experimental framework for the analysis of functional variability of neurons.



Disclosures: **F. Dos Santos Brandao:** None. **P. Andras:** None.

Poster

507. Oscillations and Synchrony: Other II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 507.30/Q3

Topic: B.07. Synaptic Transmission

Support: NISTP T32

Title: Paired recordings of pyramidal cells in the subiculum reveal local excitatory microcircuits

Authors: *M. P. FISKE, G. MACCAFERRI;
Northwestern Univ. Feinberg Sch. of Medicin, Chicago, IL

Abstract: Information exchange between neurons is accomplished using sequences of action potentials that result from the integration of local microcircuits. Unraveling the connectivity of these microcircuits and how they contribute to network activity is vital for understanding how information is relayed through the brain. Interestingly, despite its role as the main output region of the hippocampus, the microcircuity of the subiculum remains understudied. Additionally, recent evidence suggests that the subiculum is involved in generating both interictal and ictal activity in epileptic patients, providing impetus to study how these microcircuits contribute to disease. Most work involving the subiculum has focused on the excitable properties of the constituent pyramidal cells, which can be classified as either regular spiking or bursting. However, little is known about the regional synaptic connectivity. We sought to physiologically and anatomically characterize the excitatory connections of the subiculum at the individual neuron level. Using paired whole cell recordings, we have shown significant levels of connectivity between the principal cells of the subiculum. Connections were observed between bursting to bursting, regular to regular, bursting to regular, and regular to bursting neurons. These synaptic connections are excitatory and mediated by AMPA receptors at resting potential. The EPSP kinetics were similar between connection patterns, but the connection probability was highest when bursting cells were the post-synaptic target. Additionally, anatomical reconstruction of recorded cells allowed us to map the location of putative synapses. Ultimately, this work will provide insight into the population dynamics of the subiculum, which is vital for understanding the physiology of the subiculum and its role in epilepsy.

Disclosures: **M.P. Fiske:** None. **G. Maccaferri:** None.

Poster

508. Epilepsy: In Vivo and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 508.01/Q4

Topic: B.11. Epilepsy

Title: Temporal lobe epileptogenesis affects motor cortical spine dynamics *In vivo*

Authors: *C. SCHUBERT, A.-K. GELLNER, J. REIS, B. FRITSCH;
Dept. of Neurol., Albert Ludwigs Univ. Freiburg, Freiburg, Germany

Abstract: Changed dynamics of dendritic spines, as a reflection of microstructural plasticity within the hippocampus [HC], have been reported during temporal lobe [TL] epileptogenesis. However, little is known about structural remote effects on the synaptic level. Here we investigated the effects of kindling epileptogenesis in the HC on spine dynamics in the primary motor cortex [M1] longitudinally by 2-photon microscopy [2PM]. As earlier studies indicate a role of brain derived neurotrophic factor [BDNF] in the development of TLE and in synaptic plasticity we further aimed to reveal its involvement in the observed remote effects.
Combined Thy1-GFP positive and BDNF heterozygous knock out [KO] mice with a 50% reduction in neuronal BDNF and their wildtype [WT] littermates were surgically equipped with a kindling electrode in the right olfactory bulb and a chronic cranial window over the left M1. After 2 weeks of recovery the afterdischarge threshold was determined and animals were stimulated daily until the 10^{th} generalized seizure [GS]. Motor performance tests (Rotarod, Adhesive Removal Test [ART]) were performed at baseline and after the 10^{th} GS. Dendrites in layer II/III in M1 were imaged by 2PM before (baseline), during kindling (after 1^{st} stimulation, 1^{st} GS, 10^{th} GS) and 2 weeks without seizures after the 10^{th} GS.
The previously described delay in amygdala kindling epileptogenesis was also present in olfactory bulb kindling in BDNF KO mice. 2PM data of the M1 of kindled mice show a reduction of spine density during kindling epileptogenesis compared to their sham controls regardless of the genotype with a tendency towards normalization 2 weeks after the last GS. During the kindling process spine survival of pre-existing spines was significantly lower in both kindled groups compared to their sham. Only in WT the turnover ratio was significantly increased during the kindling process. Motor performance evaluated as removal time in the ART was impaired in the BDNF deficient kindled group.
We present temporal lobe epileptogenesis associated remote effects on M1 dendritic spine dynamics by longitudinal in vivo imaging. Overall temporal lobe epileptogenesis appears to result in reduced connectivity within the motor network, representing a potential mechanism underlying cognitive deficits in epilepsy patients. BDNF appears to be involved but not mainly responsible for these alterations.

Disclosures: **C. Schubert:** None. **A. Gellner:** None. **J. Reis:** None. **B. Fritsch:** None.

Poster

508. Epilepsy: In Vivo and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 508.02/Q5

Topic: B.11. Epilepsy

Title: Telemetric analysis of the dentate gyrus using the lithium/pilocarpine model of acquired epilepsy provides evidence for the progressive nature of epileptogenesis

Authors: *Z. Z. SMITH[1], F. E. DUDEK[2], D. S. BARTH[1];
[1]Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO; [2]Dept. of Neurosurg., Univ. of Utah Sch. of Med., Salt lake City, UT

Abstract: Epilepsy is a chronic disorder characterized by recurrent unprovoked seizure activity. The most common type being Temporal Lobe Epilepsy (TLE) resulting from an acute brain insult such as a Traumatic Brain Injury (TBI), infection, ischemia, or status epilepticus (SE). Pharmacological treatments produce a variety of adverse side-effects and do not adequately control seizures in over half of patients with TLE. While acquired epilepsy is commonly thought of as a progressive disorder, surprisingly little is known about the progression of the disease. Importantly, the apparent seizure free "latent period" prior to the development of spontaneous seizures may represent a therapeutic window of opportunity to halt epileptogenesis before it becomes intractable. The lack of understanding of disease progression means that there are no measures of disease risk or drug efficacy other than self-reported behavioral seizures. In order to tackle these issues, we aimed to chart the entire time course of epileptogenesis in an animal model using hippocampal and cortical 24/7 video EEG combined with a variety of pattern recognition techniques supported by complete visual analysis. We found that when using a modified lithium/pilocarpine model of epilepsy that aimed to enhance animal survivability while standardizing an acute brain insult, we could produce a variable epileptogenic response that accurately recapitulates disease progression in humans. We show that no measure of insult severity accurately predicts epileptic outcome. Moreover we show that non-convulsive seizures in our animals are visually indistinguishable from and highly prevalent prior to the development of convulsive seizures. Further analysis of hippocampal spectral electrographic signatures reveals only subtle differences between the two classes of seizures. Isolating seizure events, extensively monitoring behavior, and quantifying field potential activity around seizures allows for an accurate measure of ictal associated brain activity that provides much needed depth to the analysis of a seizure. Understanding the temporal progression of epileptogenesis and establishing targeted end points may help elucidate the underlying mechanisms of seizure disorders and aid in the development of anti-epileptic strategies.

Disclosures: Z.Z. Smith: None. **F.E. Dudek:** F. Consulting Fees (e.g., advisory boards); Epitel, though this work did not use those devices. **D.S. Barth:** None.

Poster

508. Epilepsy: In Vivo and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 508.03/Q6

Topic: B.11. Epilepsy

Support: NIH Grant R155088776

Title: An acute seizure impairs the long-term retention of a new hippocampal-dependent memory

Authors: *A. HOLLEY[1], M. S. BINDER[2], J. N. LUGO[1];
[1]Psychology and Neurosci., [2]Psychology & Neurosci., Baylor Univ., Waco, TX

Abstract: Introduction. Recent reports have indicated that a single short seizure can impair the retention of learning in delay fear conditioning. However, it is not clear how long-lasting the memory impairment can occur. The current study seeks to assess memory impairment at 24 hr and 1 wk following an acute seizure in C57BL/6 mice.
Methods. We placed 6 wk old male C57BL/6 mice into an inhalation chamber and infused flurothyl into the chamber until a generalized (tonic-clonic) seizure occurred. Control animals were run concurrently in a second inhalation chamber, but not exposed to flurothyl. An hour later mice were trained in trace fear conditioning. The mice were then tested 24 h and 1 wk later for cued recall. To assess changes to locomotor activity following a seizure another cohort of mice was tested in a 10 min open field test 24 h and 1 wk after seizure induction.
Results. A two-way ANOVA indicated a main effect of time for the 24-hour cued recall test ($p < .0001$), however no significant difference between the control and seizure groups ($p = .1763$). The 1 week follow up test showed a significant main effect of group ($p < .01$) and an effect of time ($p < .0001$) and indicating that there was a significant difference in memory retention at this time point. Results from the open field indicated a significant suppression of locomotor activity at 24 h, but not 1 wk. There were no differences between groups in horizontal movement, time spent in the center versus surround, stereotypy, rearing, or clockwise and counterclockwise revolutions.
Discussion. There was significant impairment of a marked difference in cued recall 1 wk after a seizure, but not at 24 h. Together this data suggests that there may be a period after a seizure in which new learning can be retained, but that hippocampal-dependent memory may be impaired as time passes following the seizure.

Disclosures: A. Holley: None. **M.S. Binder:** None. **J.N. Lugo:** None.

Poster

508. Epilepsy: In Vivo and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 508.04/Q7

Topic: B.11. Epilepsy

Support: NIH Grant R15SO88776

Title: Spectrographic analysis of the acute behavioral impact of early-life seizures on ultrasonic vocalizations in 129SvEvTac and C57BL/6 mice

Authors: *C. REYNOLDS, S. O. NOLAN, J. HUEBSCHMAN, J. N. LUGO, Jr;
Dept. of Psychology & Neurosci., Baylor Univ., Waco, TX

Abstract: Seizure activity during early development is known to cause long-term deficits in social behavior, learning, and memory. However, little is known regarding the acute behavioral impact of early-life seizures. Ultrasonic vocalization (USV) recordings have recently been developed as a tool for investigating early communicative deficits in mice. Previous investigation from our lab found that seizures on postnatal day (PD) 10 cause male-specific suppression of 50-kHz USVs on PD12 in 129 SvEvTac mice pups. The present study aims to extend these findings by examining putative differences in spectrographic characteristics of USVs following kainic acid-induced seizures in mouse strains with known resistance (129SvEvTac) and sensitivity (C57BL/6) to the drug. On PD10, pups were administered intraperitoneal injections of 0.5% kainic acid (2.5mg/kg), or an equivalent dose of 0.9% physiologic saline. On PD12, pups were removed from the home cage while isolation-induced recordings were captured by a broad-spectrum ultrasonic microphone and Avisoft Automated Recording Software. All recordings were converted to spectrograms by Fast Fourier Transformation (FFT) and analyzed using SASLab Pro (Avisoft Bioacoustics, Germany). These studies enhance existing evidence for neonatal vocalization behavior as an indicator of select communicative impairment in neonatal mice. In addition, this investigation provides the first known spectrographic characterization of USVs following early-life seizures.

Disclosures: **C. Reynolds:** None. **S.O. Nolan:** None. **J. Huebschman:** None. **J.N. Lugo:** None.

Poster

508. Epilepsy: In Vivo and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 508.05/Q8

Topic: B.11. Epilepsy

Support: VA grant

Title: Hippocampal glutamate neurotransmission control using optogenetics and its effects on epileptogenesis

Authors: F. POMERLEAU[1], R. ALCALA[2], P. HUETTL[1], Y. AI[1], J. JAKOBSSON[4], G. A. GERHARDT[1], *J. T. SLEVIN[3];
[1]Dept of Anat. & Neurobio., [3]Dept Neurology, L-445, [2]Univ. Kentucky, Lexington, KY; [4]Mol. Neurogenetics, Lund Univ., Lund, Sweden

Abstract: Studies that establish direct causality between neuronal activity and behavior remain scarce. Optogenetics holds the promise of possibly bridging this gap. This technique, which introduces light sensitive proteins (opsins) into neurons to regulate transmembrane ion conductance, has evolved into a salient tool for targeted control of neural systems. Using optogenetics, we have shown the feasibility of directly measuring glutamate release *in vivo* while controlling neurotransmission in the hippocampus of anesthetized animals. We hypothesized that optical modulation of hippocampal neurotransmitter dynamics would affect the complex process of epileptogenesis during amygdalar kindling. We infused (1 µl/each) AAV5-Syn-ChR2-EYFP (blue light (488 nm) induces depolarization) or AAV5-Syn-NpHR-EYFP (yellow light (594 nm) induces hyperpolarization) into the right dentate gyrus (DG) of the hippocampus. Histological analysis using yellow fluorescence revealed that, 5 weeks post-infusion, EYFP was present throughout the right hippocampus; there was some evidence of bilateral distribution. We studied 3 groups of animals: 1) ChR2-animals implanted with an optical fiber (200 µm o.d.) directly into the DG, 2) NpHR-animals implanted with electrodes in the amygdala and optical fiber in the DG and 3) sham-animals with the similar implants. Using blue light activation (488 nm, ~50 mW, 20 secs, twice a day), we kindled the ChR2 animals to Racine stage 5 seizures after ~20 stimuli. In the other groups, using yellow light activation (594 nm, ~40 mW) beginning 5 seconds prior to electrical stimulation of the amygdala (100-800 µA; 1 sec) and continuing throughout the evoked seizures, NpHR animals required more stimulations to achieve stage 4/5 seizures (20.5 ± 5.4 [SD]) than sham animals (8.2 ± 3.1 [SD]). These preliminary data suggest optogenetic manipulation of hippocampus can both induce epileptogenesis for discrete analysis of molecular and network changes and delay the epileptogenesis of amygdalar kindling.

Disclosures: F. Pomerleau: None. **R. Alcala:** None. **P. Huettl:** None. **Y. Ai:** None. **J. Jakobsson:** None. **G.A. Gerhardt:** None. **J.T. Slevin:** None.

Poster

508. Epilepsy: In Vivo and Behavior

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Topic: B.11. Epilepsy

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VA Merit Award - Peyman Golshani

DGSOM Dean of Research Fund and Departmental Funds - Peyman Golshani

NIMH Grant RO1MH101198

Title: *In vivo* interneuron circuit dysfunction in chronically epileptic mice

Authors: *T. SHUMAN[1], D. AHARONI[1], J. TAXIDIS[1], M. JAVAHERIAN[1], C. KABA[1], D. J. CAI[1], K. CHENG[1], S. E. FLORES[1], J. HODSON[1], N. RAO[1], A. FARIBORZI[1], J. LOU[1], J. DANESHRAD[1], C. YANG[1], S. GHIAEE[1], R. MANAVI[1], M. SHTRAHMAN[1], K. BAKHURIN[1], M. A. HOWARD[2], S. C. BARABAN[2], S. MASMANIDIS[1], P. GOLSHANI[1]; [1]Univ. of California Los Angeles, Los Angeles, CA; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: Epilepsy causes dramatic cell death and reorganization of interneuron circuits in both humans and rodent models but the consequences of these changes on hippocampal processing remain unknown. We used silicon probes to record single units and local field potentials throughout dorsal CA1 and dentate gyrus in head-fixed epileptic and control mice running through a virtual linear track. We found dramatic alterations of hippocampal synchronization in chronically epileptic mice, at least two months after pilocarpine-induced status epilepticus. Epileptic mice had reduced theta and gamma power and coherence, altered cross-frequency coupling, and altered phase preferences to ongoing theta oscillations. In particular, dentate hilar neurons had a similar magnitude of theta phase modulation of their firing rate, but the preferred phase of these cells as a group was more dispersed and shifted to a later phase of theta. Together, these findings indicate a significant desynchronization within and between the CA1 and dentate gyrus regions of the hippocampus that may lead to cognitive dysfunction in chronically epileptic mice. To further examine contextual information processing in these animals we recorded CA1 place cells in epileptic and control mice using custom-made head-mounted miniature microscopes to image calcium transients in large populations of CA1 neurons as animals ran across a real-world linear track over several days. We found that place fields in chronically epileptic mice were larger and contained less spatial information than in control mice. Finally,

we transplanted embryonic interneuron precursors from the medial ganglionic eminence (MGE) into the hippocampus and are testing whether these transplants, which rescue seizures and cognitive deficits in epileptic mice, can also rescue the electrophysiological and contextual processing deficits observed in epileptic mice. We predict that transplantations will restore specific deficits in hippocampal synchronization and place cell dynamics reflecting rescue of hippocampal processing.

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Poster

508. Epilepsy: In Vivo and Behavior

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Program#/Poster#: 508.07/Q10

Topic: B.11. Epilepsy

Support: COMPETE Strategic Progect UID/NEU/04539/2013

Title: Simultaneous recording of neurometabolic changes and local field potential related currents in rodent models of Epilepsy.

Authors: *A. LEDO[1,2], C. F. LOURENÇO[1], J. LARANJINHA[3], P. HUETTL[4], F. POMERLEAU[4], R. M. BARBOSA[3], G. A. GERHARDT[5];
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Abstract: Temporal lobe epilepsy (TLE) is a form of acquired epilepsy displaying recurrent seizures arising from one or both lobes of the brain. Extreme changes in neuronal excitability perturb energetic demand (initial decrease in focal O_2) and produce a vascular response (increased cerebral blood flow, CBF, and volume). The spatiotemporal dynamics of neurovascular and neurometabolic coupling during the evolution of an ictal event are poorly understood. The measurement of changes in interstitial [O_2] is attractive as it reflects both neurometabolic and vascular function. *In vivo* electrochemistry combining fast electrochemical

techniques (amperometry) with multisite microelectrodes allows us to directly measure interstitial [O_2] in the brain with high spatiotemporal resolution. The ability to record changes in brain electrical activity simultaneously and using a single electrode is particularly attractive, allowing correlations to be established between electrical activity and changes in neurometabolic and neurovascular coupling with unmatched spatial and temporal resolutions. Here we investigate changes in O_2 in the hippocampus of **awake-behaving** pilocarpine-treated rats to induce TLE. We show that seizure activity is accompanied by drastic changes in O_2. Seizure onset is accompanied by increased O_2 consumption followed by increase in interstitial [O_2] resulting from typical increase in CBF at the epileptic focus. Furthermore, the use of a microelectrode to measure changes in local field potential related currents in awake behaving rats allowed us to confirm seizure activity through the evolution of frequency power during seizure progression with a good correlation with scoring seizure intensity as well decrease with pharmacological control of seizure activity.In summery, we present a novel approach to the study of neurometabolic events associated with epileptic seizure activity with potential to be translated into the clinical setting, for example in the localization of epileptic foci in preparation for resection surgery.

Disclosures: **A. Ledo:** A. Employment/Salary (full or part-time): Quanteon, LLC, BrainSense, Lda.. **C.F. Lourenço:** None. **J. Laranjinha:** None. **P. Huettl:** None. **F. Pomerleau:** None. **R.M. Barbosa:** None. **G.A. Gerhardt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Quanteon, LLC..

Poster

508. Epilepsy: In Vivo and Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 508.08/Q11

Topic: B.11. Epilepsy

Support: 2R44AA020676-03

Title: Brain oxygen changes precede electrophysiological and behavioral signals during seizures, a new approach for epilepsy research

Authors: *J. CHANG, D. WOODWARD;
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Abstract: Electrophysiological methods, including EEG, (local field potentials) LFP and single unit recordings, have been mainstream approaches for epilepsy research in decades. Recent

development of imaging technology is a significant addition to the repertoire of research tools. However, restrictions by most instrumentation for imaging studies do not allows real time monitor of epileptic seizures during freely moving conditions. Recent studies demonstrated that real time brain oxygen measurement can be a proxy for fMRI in freely moving rats (Lowry 2010). We have developed 8 carbon fiber electrode arrays to record both O_2 transients and LFP signals in freely moving rats subject to kindling and chemical induced seizures. Brain tissue O_2 content was measured by fast scan cyclic voltammetry (FSCV) using a 6 carbon fiber electrode array. LFP were recorded using two adjacent carbon fiber electrodes at the same location. Simultaneous behavioral responses were recorded by a video camera. This experimental setting allows measurement of metabolic, electrophysiological and behavioral signals simultaneously in freely moving rats in different models of epilepsy. In an amygdala kindling model, subthreshold kindling at 25 uA induced a clear increase in oxygen concentration, lasting 3-10 s in dorsal hippocampus and nucleus accumbens without detecting behavioral and LFP responses. Continuous kindling at high current induced stage 5 seizures accompanied by both the increase of O_2 content and electrophysiological seizures. In chemical induced seizures, pentylenetetrazole was injected (50mg/kg, i.p.) and convulsive seizures developed within 2 min. An initial increase in oxygen concentration appeared well before the LFP and behavioral seizures became evident during this 2 min window. At this point the earliest event leading to seizure is found to be a local increase in oxygen concentration in limbic regions. This appears before detection of both electrical and behavioral signs of seizure. Preliminary data show an increase in local oxygen with spontaneous seizures induced by status epilepticus in temporal lobe epilepsy model. These experiments demonstrate that use of distributed sensors of real time transient changes in tissue oxygen is a promising new tool for study of events predicting seizures that will complement traditional electrical and behavioral measures.

Disclosures: J. Chang: None. **D. Woodward:** None.

Poster

508. Epilepsy: In Vivo and Behavior

Location: Halls B-H

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Program#/Poster#: 508.09/Q12

Topic: B.11. Epilepsy

Title: A behavior-based screen for the identification of novel anti-epileptic drugs

Authors: *T. EVRON[1], A. VELENICH[1], T. Z. DEEB[2], Q. WANG[3], D. BAKER[4], N. J. BRANDON[3], S. MOSS[2], D. KOKEL[5], R. T. PETERSON[6];
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Neurosci., Boston, MA; [3]AstraZeneca Neurosci. iMED, Cambridge, MA; [4]AstraZeneca, Cambridge, United Kingdom; [5]Inst. for Neurodegenerative Diseases, UCSF, San Francisco, CA; [6]Cardiovasc. Res. Center, MGH, Charlestown, MA

Abstract: Epilepsy is a spectrum of neurological disorders, characterized by recurrent seizures. The available anti-epileptic drugs (AEDs) control seizures in about two thirds of epilepsy patients but leave a third poorly treated, creating demand for new therapeutic strategies. Phenotypic behavior-based screens are powerful and effective tools for CNS drug discovery and could potentially lead to identification of novel AEDs. We have developed a unique platform for *in vivo* high throughput and behavior-based screens in zebrafish. The platform is a high content imaging system that combines robotic stimulus presentation in 96-well format with high-quality digital video capture and image processing algorithms, allowing us to profile the behavioral responses of zebrafish to small molecules. Using this platform we modeled epileptic-like activity in zebrafish larvae using the potassium chloride co-transporter KCC2 inhibitor VU0463271. Because KCC2 loss-of-function has been associated with epilepsy in humans, we screened for suppressors of the VU0463271-induced epileptic-like behaviors. We identified two compounds, TT0023831 and TT0042607 that reverse the VU0463271-induced effect in zebrafish. In addition, TT0042607 reverses epileptic-like behaviors induced by the GABA antagonist pentylenetetrazole (PTZ) and both compounds reduce excitability in rat cultured hippocampal neurons, as evidenced by reduced firing rates and delayed spike initiation. These compounds are compelling starting points for ongoing drug discovery efforts including lead optimization and validation in mammalian epilepsy models.

Disclosures: **T. Evron:** A. Employment/Salary (full or part-time): Teleos Therapeutics, LLC. **A. Velenich:** A. Employment/Salary (full or part-time): Teleos Therapeutics, LLC. **T.Z. Deeb:** None. **Q. Wang:** A. Employment/Salary (full or part-time): AstraZeneca. **D. Baker:** A. Employment/Salary (full or part-time): AstraZeneca. **N.J. Brandon:** A. Employment/Salary (full or part-time): AstraZeneca. **S. Moss:** F. Consulting Fees (e.g., advisory boards); AstraZeneca, SAGE Therapeutics. **D. Kokel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Teleos Therapeutics, LLC. **R.T. Peterson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Teleos Therapeutics, LLC.

Poster

508. Epilepsy: In Vivo and Behavior

Location: Halls B-H

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Program#/Poster#: 508.10/Q13

Topic: B.11. Epilepsy

Support: NIH Grant r01ns071153

Title: The role of tau in modulating hyper-excitability in an emerging model of tumor associated epilepsy

Authors: *A. HATCHER[1], K. YU[2], J. LALONDE[3], B. DENEEN[2], J. NOEBELS[3];
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Abstract: Hyper-excitability of neuronal networks underlies the pathophysiology of epilepsy, and is known to be a component of other neurological disorders including cancers of the brain. Previous work has shown that genetic removal of neuronal microtubule organizer and stabilizer *tau* can significantly reduce epileptiform activity and lethality across a number of models of hyper-excitability, including Alzheimer's Disease models, the Kcna1 early lethal mouse model of epilepsy, and the Scn1a mouse model of Dravet syndrome. We recently confirmed a progressive seizure phenotype in a novel transgenic mouse model of brain tumor associated epilepsy. The effect of *tau* loss on hyper-excitability and lethality in this glioblastoma model is unknown. To answer this question, we generated glial derived brain tumors on a *tau* knockout background using a CRISPR *in utero* electroporation strategy. Using chronic video electroencephalography (EEG), we recorded cortical inter-ictal spike and seizure activity in these tumor mice at various timepoints in disease progression, monitored survival, and assessed tumor burden via immunohistochemically methods. In preliminary results, *tau* KO tumor mice exhibited reduced inter-ictal spike activity and slightly longer survival margins compared to *tau* WT tumor-bearing littermates. These data suggest that *tau* loss may provide a modest protective effect in this model of cortical hyper-excitability, and further investigation is under way to confirm these initial findings.

Disclosures: A. Hatcher: None. **K. Yu:** None. **J. Lalonde:** None. **B. Deneen:** None. **J. Noebels:** None.

Poster

508. Epilepsy: In Vivo and Behavior

Location: Halls B-H

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Program#/Poster#: 508.11/Q14

Topic: B.11. Epilepsy

Title: Oriental medicine Woohwangchungsimwon attenuates kainic acid-induced seizures and neuronal cell death in the hippocampus

Authors: *J. CHOI[1], M. JANG[2], I.-H. CHO[2];
[1]Col. of Korean Medicine, Kyung Hee Univ., Seoul, Korea, Republic of; [2]Dept. of Convergence Med. Sci., Col. of Korean Medicine, Kyung Hee university, Seoul 02447, Korea, Republic of

Abstract: Woohwangchungsimwon (WCW) is an oriental medicine that has been extensively prescribed in Asia to patients with apoplexy, high blood pressure, acute/chronic convulsion, etc. However, the potential therapeutic value of WCW in treating the pathologic brain has not yet been fully investigated. In the present study, we evaluated whether WCW has beneficial effects on kainic acid (KA)-induced excitotoxicity. An intraperitoneal injection of KA (40 mg/kg) and an intracerebroventricular (i.c.v.) injection of KA (0.2 μg) produced typical seizure behavior and neuronal cell death in the CA1 and CA3 pyramidal layers of the hippocampus, respectively. However, the systemic administration of WCW significantly attenuated the seizure behavior and neuronal cell death. WCW was found to exert the best protective effect when it was administrated 2 hours before a KA-injection. Moreover, this WCW-induced neuroprotection was accompanied by a reduction in microglia activation and tumor necrosis factor-alpha, interleukin (IL)-1beta, IL-6, heme-oxygenase-1, inducible nitric-oxide synthase, and cyclooxyganase-2 in the hippocampus. These results suggest that WCW has therapeutic potential to suppress KA-induced pathogenesis in the brain by inhibiting inflammation.Rejuvenation Res. 2016 Mar 16. [Epub ahead of print]

Disclosures: **J. Choi:** None. **M. Jang:** None. **I. Cho:** None.

Poster

508. Epilepsy: In Vivo and Behavior

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Program#/Poster#: 508.12/R1

Topic: B.11. Epilepsy

Support: Seed Money Grant, The Aga Khan University

Title: Australian parrots have lower threshold for PTZ-induced myoclonic jerks compared to sparrows

Authors: *F. M. ARAIN[1], F. AMIN[2], A. H. DAR[2], F. RAGHIB[2], G. HAIDER[2];
[1]Neurosci., [2]Biol. and Biomed. Sci., The Aga Khan Univ., Karachi, Pakistan

Abstract: Epilepsy is common disorder that affects over 50 million people worldwide. It is characterized by occurrence of two or more unprovoked seizures. Despite extensive research and discovery of several treatment options, seizures remain refractory in 30% of patients. This long-standing poor response to the available treatment options indicate that the current research models are not sufficient and new models need to be discovered in order to better understand this disease. Mammals have been traditionally used to study epilepsy. Aves remain a promising yet largely under explored model of epilepsy. In this study we compared the response of two species of birds, Australian Parrots (APs) and Sparrows (SPs), to a pro-epileptic drug, Pentylenetetrazole (PTZ). First, APs and SPs were injected with saline intraperitoneally to get accustomed to handling and injecting. After 30 minutes the birds were injected with 25mg/kg, 50mg/kg and 75mg/kg PTZ or saline. The behavior of birds was video recorded and analyzed by two observers. Myoclonic jerks (MJ) and tonic clonic seizures (TCS) were observed in both species however unique behavioral changes in response to PTZ, including tonic posturing, intermittent rapid alternating neck movement, biting behavior and head bobbing, were only seen APs. The frequency of MJ in APs was greater at the dose of 75 mg/kg (3.84 ± 1 MJ/min) compared to both 50 mg/kg (0.42 ± 0.1 MJ/min) (p value < 0.005) and 25 mg/kg (0.49±0.1 MJ/min) (p value < 0.006). In SPs the frequency of MJ was not significantly different at the dose of 75 mg/kg (1.21 ± 0.49 MJ/min) compared to 50 mg/kg (0.17 ± 0.1 MJ/min) (p value < 0.134) or 25 mg/kg (0 ± 0 MJ/min) (p value < 0.085). The frequency of MJ was greater in APs as compared to SPs at 25 mg/kg (p value <0.016) and 75 mg/kg (p value < 0.034). APs also had significantly reduced latency of onset of MJ compared to SPs at 25 mg/kg (p value < 0.001) and 50 mg/kg (p value < 0.004). Interestingly SPs had a reduced latency to TCS as compared to APs at the dose of 75 mg/kg (p value < 0.037). These findings indicate that APs have a significantly greater frequency and lower threshold of onset of MJ and induction of unique behavioral changes in response to PTZ treatment as compared to SPs. Interestingly SPs have reduced latency of TCS as compared to APs, indicating SPs are more predisposed to directly developing a TCS. Further studies need to be conducted to identify the reason for these variations in responses of APs and SPs to PTZ. As it is known that PTZ is an inhibitor of GABA, future studies should be conducted to identify if difference in regional and total expression of GABAergic and Glutamatergic neurons and GABA receptors occurs in APs and SPs.

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Poster

508. Epilepsy: In Vivo and Behavior

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Topic: B.11. Epilepsy

Support: Ministerio de Economía y Competitividad (BFU2015-66887-R)

Title: Role of sharp-wave fast ripples in deficits of episodic-like memory in temporal lobe epilepsy

Authors: *M. VALERO[1], R. G. AVERKIN[2], D. LOPEZ-PIGOZZI[1], J. R. BROTONS-MAS[1], E. CID[1], L. M. DE LA PRIDA[1];
[1]Inst. Cajal - CSIC, Madrid, Spain; [2]Dept. of Physiology, Anat. and Neurosci., MTA-SZTE Res. Group for Cortical Microcircuits, University of Szeged, Hungary

Abstract: Hippocampal sharp wave (SPW)-ripple (100-200 Hz) complexes emerge during immobility and slow-wave-sleep to regulate single-cell activity in memory consolidation. In temporal lobe epilepsy (TLE) SPW-ripples are faster (200-600 Hz) so called fast-ripples (SWP-FR). Despite their clinical value as epileptogenic biomarkers, the cognitive implications of SPW-FR remain unknown. To address this issue, we evaluated SPW events recorded with multi-site silicon probes during offline processing in rats experiencing an object-recognition episodic-like memory task. SPW-FRs recorded during immobility and slow-wave sleep in TLE animals were characterized by a stronger contribution above the normal ripple band (>250 Hz, FR-index) in association with increased spectral entropy. Interestingly, the FR-index correlated specifically with the rat's ability to discriminate the temporal component of episodic-like memory. Moreover, consolidation of a spatial memory was impaired in TLE rats when tested at 100 min versus 50 min interval in a simplified version of the task. We looked for the mechanisms of this disruption at the single-cell level with juxtacellular recording and labeling of CA1 hippocampal cells in freely moving rats. Single-cells recorded from epileptic rats were more desynchronized and positively modulated during SPW-FR than control cells during ripples. Applying methods of clustering analysis to SPW-ripple events we observed a high firing selectivity for certain ripple-waveforms in control cells, whereas epileptic cells fired more and indistinctly over all groups. We propose that SPW-FR act to disorganize hippocampal activity during memory consolidation processes.

Disclosures: M. Valero: None. **R.G. Averkin:** None. **D. Lopez-Pigozzi:** None. **J.R. Brotons-Mas:** None. **E. Cid:** None. **L.M. de la Prida:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 509.01/R3

Topic: B.12. Glial Mechanisms

Support: NIMH Ko1 MH087845

Brain Behavior Research Foundation Young Investigator Award

Title: Inactivation of fgfr1 and fgfr2 in postnatal astrocytes

Authors: *L. RUBIN, K. SMITH, C. BARNETTE, D. ROGERS;
Biol., Univ. of Louisiana At Lafayette, Lafayette, LA

Abstract: Fibroblast growth factors (Fgfs) are a family of 22 cytokines that bind to 4 receptors, many of which play a critical role in cortical development. Fgf ligands, including Fgf2, and the Fgfr1 and Fgfr2 receptors are expressed by astrocytes and astrocytic stem cell lineages of the developing and adult CNS. Previous studies have shown that Fgfrs can have compensatory effects on proliferation and development. We have inactivated Fgfr1 and Fgfr2 in postnatal astrocytes by tamoxifen inducible Cre mediated recombination using the hGFAP-CreERT2 (GCE) transgene. We targeted postnatal astrocytes by administering injections of tamoxifen from P14-17, 60 mg/kg i.p. We tested locomotor behavior of the mice for 30 minutes in an open field. Male double KO mice showed hypoactivity compared to male control littermates with total time immobile having a p-value of .0380. Other trends were less distance travelled (p=.0542), lower mean speed (.0567), and more total mobile episodes (0521) possibly indicating that the animals began more movements but were slower and spent more time resting. We observed no differences in anxiety behaviors on the elevated plus maze test, and no differences in memory were observed in the 1-day morris water maze. It was previously shown that Fgfr1 and Fgfr2 double KO starting at E13.5 lead to multiple cerebellar abnormalities. The locomotor behavior findings lead us to hypothesize that there may be a postnatal cerebellar defect in Fgfr1/Fgfr2 double KO mice. We compared control and Fgfr1/Fgfr2 double KO mice on a hindlimb clasping test. We found double KO mice had significantly higher scores on this test (p=.05), indicating an impairment in motor coordination. Future work will examine cerebellar morphology. We will compare the effects of Fgfr1 single and Fgfr1/Fgfr2 double mutants upon PV neuron maturation, and postnatal hippocampal proliferation.

Disclosures: L. Rubin: None. **K. Smith:** None. **C. Barnette:** None. **D. Rogers:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: B.12. Glial Mechanisms

Support: NSF 1353739

Microscopy Society of America

University of Arizona Honors College

NSF Western Alliance to Expand Student Opportunities

private foundation

Title: *Drosophila* astrocytes span functional neural domains

Authors: *E. HERNANDEZ[1], S. E. MACNAMEE[2], L. R. KAPLAN[1], J. A. CHARLTON[1], D. S. FARHADI[1], K. N. LANCE[2], L. P. TOLBERT[2], L. A. OLAND[2];
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Abstract: Ignorance of the contribution of glial cells limits our understanding of how neuronal circuits function. Astrocytic processes invest synaptic neuropil at high density and astrocytes both respond to and modulate neuronal activity, even in invertebrates. *Drosophila,* with its extraordinary genetic toolkit, can be exploited to probe the contribution of astrocytes to neuronal circuit function. In the current study, we ask whether astrocytic arbors map specifically onto functional neuropilar domains (e.g., sensory, motor) or more broadly span across domains in *Drosophila*. We also ask how individual astrocytes interface with neighboring astrocytes.
We are analyzing astrocytes in detail, both individually and as parts of the complex neuron-glia network, in the segmented ventral nerve cord (VNC) of the third-instar larva. Of the 12 astrocytes present in each of the repeating segments, 4 can be reliably identified by the position of their cell bodies. Our recent characterization of the electrophysiological and morphological features of the VNC astrocytes demonstrated strong similarities to vertebrate astrocytes: low membrane resistance and high capacitance, passive membrane properties, expression of the EAAT1 glutamate transporter, and limited dye-coupling (MacNamee et al., J Comp Neurol 2016). Here we use Flp-out (Ito et al., 1997) and MultiColor FlpOut (Viswanathan et al., 2015) genetic constructs targeted to astrocytes via the *alrm*-GAL4 driver (Doherty et al., 2009) to generate high-resolution, detailed images of these astrocytes. Our examination of the processes of individual astrocytes as well as the interfaces between adjacent astrocytes has revealed that (1) the arbors of individual astrocytes span multiple functional neural domains and (2) astrocytic branches have convoluted spatial domains, in which the finest distal branches of adjacent astrocytes interweave with a variable, but small, amount of interdigitation.
These findings suggest the following functional consequences: Because the arbors of astrocytes span functional domains and the astrocytes also can be dye-coupled, astrocytes may have the role of integrating neuronal activity across disparate neural circuits. Alternatively, there may be functional sub-compartments in astrocytes that are differentially active in response to local synaptic activity; this possibility remains to be explored. The limited overlap between astrocytes indicates that electrical coupling among astrocytes is likely to occur at the distal-most tips. And finally, any given synapse is likely to fall exclusively within the domain of a single astrocyte.

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Poster

509. Astrocyte Cell Biology and Modulation II

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Program#/Poster#: 509.03/R5

Topic: B.12. Glial Mechanisms

Support: BBSRC CASE studentship with Eli Lilly 1370116

Title: Mechanism of phasic D-Aspartate false-gliotransmitter release in the rodent barrel cortex

Authors: ***S. ANTONIO**[1], D. URSU[2], R. PARRI[1];
[1]Aston Univ., Birmingham, United Kingdom; [2]Eli Lilly and Co., Windlesham, United Kingdom

Abstract: Astrocytes modulate synaptic transmission, control blood flow, and extracellular ion and transmitter concentrations, and thus are of great importance in the normal and pathological brain. Astrocytes show bidirectional communication with neurons, releasing gliotransmitters (GTs) such as glutamate, ATP, and GABA that modulate synaptic and neuronal network activity. Various mechanisms of GT release have been proposed, such as via calcium dependent vesicular release, Excitatory amino acid (EAA) transporters, large pore channels, ion channels such as TREK1 and Bestrophin-1 (Best-1), and hemichannels. In this study we investigated the mechanism underlying NMDA receptor mediated slow inward current (SIC) generation in an enhanced EAA release model. Following pre-exposure to 100 μM D-Aspartate, Patch clamp recordings were conducted in layer 2/3 pyramidal neurons in rodent thalamocortical slices from animals at P10-P28. TTX-insensitive slow-inward currents (SICs)(defined as having an amplitude >20pA and rise time >20ms) were observed and pharmacological methods were used to characterise the mechanisms of release. SICs were not abolished by inhibition of VGLUT1, hemichannels, TREK1 channels, temperature or volume regulated channels, or anion channels such as Best-1. SICs still occurred in the presence of Ca^{2+} ATP-ase inhibitor cyclopiazonic acid. Similarly, there was no significant difference in SIC rate between wild-type and inositol 1,4,5-triphosphate (IP_3) type-2 receptor knock-out mice, indicating that spontaneous SIC emergence can be independent of IP_3 mediated intracellular store calcium release. Together, these results suggest that this enhanced astrocyte EAA release model is resistant to pharmacological blockade of individual efflux pathways.

Disclosures: **S. Antonio:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BBSRC CASE studentship with Eli Lilly. **D. Ursu:** A. Employment/Salary (full or part-time): Eli Lilly and Company. **R. Parri:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Topic: B.12. Glial Mechanisms

Support: BFU2012-38844 to E.G

2014SGR984 to E.G. and R.M.

Title: Highly distinct CREB-dependent transcriptional programs in astrocytes and neurons

Authors: L. PARDO[1], L. VALOR[2], A. ERASO[1], A. BARCO[3], R. MASGRAU[1], *E. GALEA[4,5,1];
[1]Inst. de Neurociencias and Departament of Bioquímica, Bellaterra, Spain; [2]Hosp. Universitario Puerta del Mar, Cadiz, Spain; [3]Inst. de Neurociencias, Alicante, Spain; [4]Univ. Autònoma de Barcelona, Bellaterra, Barcelona, Spain; [5]ICREA, Barcelona, Spain

Abstract: The cAMP response element binding protein (CREB) is a primary hub of activity-driven genetic programs in neurons controlling plasticity, neurogenesis and survival. By contrast, the gene networks coordinated by CREB in astrocytes are not yet characterized despite the fact that astrocytic CREB is also activity-driven and neuroprotective like its neuronal counterpart. To fill this gap, we characterized in six stages the transcriptional programs regulated by CREB in astrocytes as compared to neurons. First, CREB-dependent transcription was activated in primary cortical rat astrocyte cultures with noradrenaline (NE), the adenylate cyclase activator, forskolin (FSK) or a virally transduced constitutively active CREB (VP16-CREB). Second, transcriptomic changes were characterized with Agilent DNA microarrays. Third, the differentially expressed genes ($p<0.05$) were filtered through the first quartile of the translational ribosome affinity purification (TRAP) repository of adult astrocyte genes to remove embryonic signatures. Fourth, the resulting list was subjected to functional enrichment analysis with Gene Ontology (GO) using ClueGO v1.4 and ReviGo softwares. Fifth, candidates for direct CREB target genes were identified with the CREB-target gene database of the Salk Institute. Sixth, the functional and molecular CREB signatures in astrocytes were compared to the neuronal ones identified in cultured neurons stimulated with FSK or over-expressing VP16-CREB (ArrayExpress database; accession number E-MEXP-3167). We found that VP16-CREB, FSK

and NE significantly changed the gene expression profile in astrocytes. The FSK and NE groups were very similar (Pearson's correlation = 0.72), while the VP16-CREB group was markedly different from the other two (Pearson's correlation = 0.18 vs FSK; 0.21 vs NE), indicating that the chronic activation of CREB dependent transcription comprises the programs regulated by FSK and NE and activates additional programs. The ranking according to statistical significance of GO groups regulated by CREB, and of CREB candidate target genes, revealed little overlap between astrocytes and neurons. "Nuclear functions" including "Transcription" was the top hit in both cells—represented by different genes—confirming the central position of CREB in transcriptional networks. Ranked second were, as expected, "Plasticity" or "Neurotransmission" in neurons and, unexpectedly, "Mitochondrial functions" in astrocytes. The databases generated in this study will provide a tool to explore novel means whereby CREB impinges on key brain functions by coordinating gene networks and sub-organellar functions in astrocytes.

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Poster

509. Astrocyte Cell Biology and Modulation II

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Program#/Poster#: 509.05/R7

Topic: B.12. Glial Mechanisms

Support: Conacyt 79502

Title: Co-exposure to arsenic and fluoride increases Glutamate uptake in Bergmann glial cells: evidence of oxidative stress involvement

Authors: A. L. GARCÍA LÓPEZ, O. G. MÉNDES FLORES, L. DEL RAZO, *E. LOPEZ-BAYGHEN, A. ORTEGA;
Toxicology, CINVESTAV-IPN, México, Mexico

Abstract: Inorganic arsenic (iAs) and fluoride (F^-) are known neurotoxicants that are able to cause structural and functional damages in the Central Nervous System (CNS). The concomitant exposure to iAs and F^- doses commonly occur. In the cerebellum, Bergmann glial cells (BGC) enwrap the excitatory synapses established between parallel fibers of granular cells and Purkinje cells and participate in pH regulation, K^+ homeostasis, lactate supply and the Glu/glutamine shuttle. BGC express glutamate transporters of the GLAST/EAAT1 subtype that allow them to remove the excitatory signal of the synaptic cleft and by these means prevent an excitotoxic insult. To gain insight into the molecular events that can be induced upon coexposure to iAs and

F^- in these cells at the level of glutamate transporter function. we used the well established model system of cultured chick cerebellum BGC. We detected a significant increase in Glu uptake activity after 3μM iAs and 500μM F^- co-exposure. Kinetic studies revealed that the increase is related to an augmented transporter affinity. Pretreatment of the exposed cells with the antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) prevented the metalloid effect (in Glu uptake) demonstrating that reactive oxygen spices might be implicated in the molecular mechanisms involved in the described phenomena. These preliminary studies suggest that exposure to these pollutants could have the transporter EAAT1/GLAST in glial cells a molecular target of toxicity and that this effect is closely related with oxidative stress.

Disclosures: A.L. García López: None. **O.G. Méndes Flores:** None. **L. Del razo:** None. **E. Lopez-Bayghen:** None. **A. Ortega:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 509.06/R8

Topic: B.12. Glial Mechanisms

Support: NIH NS037585

NIH NS061764

Title: Wakefulness-dependent modulation of D-serine

Authors: *M. TOLMAN[1], P. G. HAYDON[2];
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Abstract: Cholinergic signaling controls brain states, modulates circuit function, and is a primary therapeutic strategy for a number of diseases including Alzheimer's disease. Recent work from our lab has demonstrated in mouse hippocampal slices that wakefulness-dependent acetylcholine release acts on alpha 7 nicotinic acetylcholine receptors (α7nAChRs) expressed by astrocytes to control the availability of the obligatory NMDA receptor (NMDAR) co-agonist, D-serine. However, wakefulness dependent changes in hippocampal D-serine levels have not been measured *in vivo.* To address this, I am using *in vivo* microdialysis and high pressure liquid chromatography (HPLC) to directly measure D-serine levels as a function of wakefulness. During periods of sustained wakefulness, we find that D-serine levels remain high in the hippocampus, but decrease when the mouse transitions to sleep. This change is observed independently of changes in L-serine and are in agreement with our previous *in situ* data. Using

cell type specific deletion and activation of the α7nAChRs, we are determining the role of cholinergic activation of astrocytes and neurons in mediating wakefulness-dependent elevations in D-serine.

Disclosures: M. Tolman: None. **P.G. Haydon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GliaCure.

Poster

509. Astrocyte Cell Biology and Modulation II

Location: Halls B-H

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Program#/Poster#: 509.07/R9

Topic: B.12. Glial Mechanisms

Support: British Heart Foundation Grant PG/13/79/30429

Marie Curie Fellowship 654691

Russian Foundation for Basic Research Grant 16-34-00159

Title: Processing of afferent information at the level of NTS: the role of astroglial 5-HT_{2A} receptors

Authors: *S. MASTITSKAYA[1], P. S. HOSFORD[1], E. TUROVSKY[2], A. V. GOURINE[1], A. G. RAMAGE[1];
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Abstract: Introduction. 5-HT (serotonin) is one of the principal transmitters implicated in central cardiovascular regulation. 5-HT_{2A} receptor activation at the level of the nucleus tractus solitarius (NTS) produces hypotension and facilitates bradycardias evoked by vagal afferents and activation of arterial baroreceptors (Ramage & Villalón, 2008, *TiPS*. 29:472-81). Recently, it has been suggested that astrocytes play an important role in cardiovascular integration at the level of the NTS (Lin et al., 2013, *J Neurosci*. 33:18608-17)**.** In this study we investigated the effects of 5-HT on NTS astrocytes and tested the hypothesis that 5-HT receptors contribute to astroglial activation in response to incoming afferent information.
Methods. Primary brainstem astroglial cultures were loaded with a Ca^{2+} indicator Fura-2, and changes in intracellular [Ca^{2+}] evoked by 5-HT were recorded using fluorescent microscopy. 5-HT agonists and antagonists were used to characterize the identity of 5-HT receptors. $[Ca^{2+}]_i$ responses in NTS astrocytes *in vivo* were visualized using a genetically encoded Ca^{2+} indicator

GCaMP6. NTS astrocytes of young male rats (~100g) were targeted to express GCaMP6 using microinjections of an adenoassociated viral vector under control of an enhanced GFAP promoter. 4 weeks later, rats were anaesthetized with α-chloralose, neuromuscular blocked and artificially ventilated. The dorsal surface of the brainstem was exposed and astroglial $[Ca^{2+}]_i$ responses evoked by electrical stimulation of the vagus nerve (5 Hz, 0.8 mA, 1 ms pulse duration) were recorded using MiCam02 high-resolution camera.
Results. Brainstem astrocytes in culture responded to 5-HT with vigorous elevations in intracellular $[Ca^{2+}]$ (EC_{50} 10 μM). 5-HT-induced $[Ca^{2+}]_i$ responses were blocked by phospholipase-C inhibitor U73122 (5 μM) and by 5-HT_{2A} antagonist ketanserin (0.01 - 1 μM). Neither 5-HT_{2C} agonist WAY161503 (0.01 - 5 μM) nor 5-HT_{2B} agonist BW723C86 (0.001 - 1 μM) had any effect on $[Ca^{2+}]_i$ in brainstem astrocytes. *In vivo*, electrical stimulation of the vagus nerve induced rapid $[Ca^{2+}]_i$ responses in the NTS astrocytes as determined by changes in GCaMP6 fluorescence ($\Delta F/F_{max}$=2.0±0.06; n=5). Intravenous administration of ketanserin (300 μg kg^{-1}) significantly ($p<0.01$) decreased the peak magnitude of $[Ca^{2+}]_i$ responses ($\Delta F/F_{max}$=1.0±0.02; n=5), indicating that 5-HT acting at 5-HT_{2A} receptors mediates (at least in part) the effects of vagal afferent evoked changes in NTS astrocytes.
Conclusions. These data suggest that 5-HT-induced modulation of cardiovascular reflexes at the level of NTS may involve its actions on local astrocytes expressing 5-HT_{2A} receptors.

Disclosures: S. Mastitskaya: None. **P.S. Hosford:** None. **E. Turovsky:** None. **A.V. Gourine:** None. **A.G. Ramage:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Program#/Poster#: 509.08/R10

Topic: B.12. Glial Mechanisms

Support: NIH Grant 5R01AG048814

LSRF Merck Research Laboratories Postdoctoral Fellowship

Title: Synapse pruning by astrocytes regulates hippocampal circuit development

Authors: *L. CLARKE[1], W.-S. CHUNG[2], B. BARRES[1];
[1]Neurobio., Stanford Univ., Stanford, CA; [2]Dept. of Biol. Sci., KAIST, Daejeon, Korea, Republic of

Abstract: During normal brain development excessive synapses are formed between neurons, and in order to refine the information processing capacity of the brain unnecessary synapses are eliminated or pruned. We have discovered that this pruning process is carried out by a class of glial cells called astrocytes. These cells express the phagocytic receptors MEGF10 and MERTK which recognize 'eat me signals' on the unwanted synapses, and by the process of phagocytosis they engulf the unwanted connections. Importantly, when astrocyte-mediated phagocytosis is impaired developmental synapse remodeling in the visual system remains incomplete, indicating that astrocytes actively promote synapse elimination. Despite this important role for visual system development, it is not known whether astrocyte-mediated synapse elimination is important for other fundamental aspects of brain function, such learning a memory, which requires both the formation of new synapses and the elimination of pre-existing synapses. To investigate the role of astrocyte-mediated synapse elimination in learning and memory, whole-cell patch-clamp recordings were performed to compare the number of functional synapses in the hippocampus of mice deficient in MEGF10 and MERTK with normal mice. Interestingly, mice deficient in MEGF10 and MERTK received three times more the number of functional excitatory synapses when compared to normal mice. In contrast, impaired astrocyte-mediated synapse elimination did not change the number of inhibitory synapses, suggesting that different refinement pathways exist for different types of synapses. After establishing a role for astrocytes in the development of hippocampal neural circuits, ongoing experiments are being performed to assess the role of astrocyte-mediated synapse elimination in *in vitro* models of learning and memory and *in vivo* hippocampal dependent behavioral tasks in adulthood. These experiments will address the question of whether astrocytes can respond to the changes in neural activity thought to underlie learning and memory and prune neural circuits to allow for long-term changes in neural connectivity.

Disclosures: **L. Clarke:** None. **W. Chung:** None. **B. Barres:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 509.09/R11

Topic: B.12. Glial Mechanisms

Title: Tubastatin A and other HDAC6 inhibitors regulate FGF21 transcript expression in glia cells

Authors: *Y. LENG[1], J. WANG[1], P. LEEDS[1], Z. WANG[1], M. PENZO[2], D.-M. CHUANG[1]; [1]Mol. Neurobiol Section, [2]Unit on the Neurobio. of Affective Memory, Natl. Inst. Mental Health/NIH, Bethesda, MD

Abstract: Histone deacetylases (HDACs) are promising therapeutic targets for CNS diseases by epigenetic regulation of gene expression. Among them, HDAC6 is exclusively located in the cell cytoplasm and involved in various neuropathological conditions. Recently, our lab reported that Tubastatin A (TubA), a potent and highly selective HDAC6 inhibitor, alleviates stroke-induced brain infarction and functional deficits possibly through α-tubulin acetylation and up-regulation of fibroblast growth factor 21 (FGF21) (Wang et al, *Scientif Rep*, Jan, 2016). FGF21, a newly determined regulator of glucose and lipid metabolism, is neuroprotective and a novel target of mood stabilizers lithium and valproate (VPA) in brain neurons (Leng et al., *Mol Psychiatry*, 2015). Moreover, VPA and other pan HDAC inhibitors upregulate FGF21 gene expression by inhibiting HDAC 2 and 3 in C6 glioma and primary glial cells (Leng et al, *Intl J Neuropsychopharm*, 2016). As an extension of previous work, this study investigated the effects of TubA and other HDAC6 inhibitors on the expression of FGF21 in C6 glioma (C6) cells. Incubation of C6 cells with TubA at concentrations of 1 to 5 μM induced a dose-dependent increase in FGF21 mRNA levels with a maximum increase of 3-fold at 5 μM. Acetylated histone H3 and acetylated α-tubulin protein levels were also markedly increased by TubA treatment. Other HDAC6-specific inhibitors, Nexturastat A and Tabacin, increased FGF21 mRNA expression dose-dependently, reaching a significant increase at 2μM, and maximum increases of 6- and 8-eight-fold, respectively, at 5 μM. Two other non-specific HDAC6 inhibitors ACY1215 and CAY10603 were found to increase FGF21 mRNA levels up to 20- or 80-fold, respectively. To investigate whether HDAC6 silencing also regulates FGF21 transcription levels, we utilized the lentiviral small hairpin RNA (shRNA)-mediated knockdown technology. In C6 cells, we tested five clones and found that HDAC6 #414 produced the most efficient knockdown (ca. 63%) of HDAC6 mRNA. FGF21 transcript level was significantly increased by 1.7-fold with lentiviral mediated HDAC6 knockdown using three pairs of different primers from Integrated DNA Technologies Inc. Similar results were confirmed using three other pairs of primers from ThermoFisher Scientific, in which FGF21 mRNA level was increased 1.5-, 1.6-, or 1.7-fold. Taken together, our novel results show that inhibition of HDAC6 by five different HDAC6 inhibitors, or lentiviral mediated shRNA knockdown, up-regulates FGF21 transcript levels in C6 glioma cells. Further studies of the neurobiological significance of HDAC6 inhibition on FGF21gene regulation in brain disease models are warranted.

Disclosures: **Y. Leng:** None. **J. Wang:** None. **P. Leeds:** None. **Z. Wang:** None. **M. Penzo:** None. **D. Chuang:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 509.10/R12

Topic: B.12. Glial Mechanisms

Support: UCLA Chancellor's Postdoctoral Fellowship

Title: Estrogen-responsiveness of prepubertal vs. adult female hypothalamic astrocytes

Authors: *M. A. MOHR, P. MICEVYCH;
Dept of Neurobiology, David Geffen Sch. of Med., UCLA, Los Angeles, CA

Abstract: Puberty is a complex developmental stage that results in reproductive maturation. In females, reproductive circuits are considered mature when estrogen positive feedback is able to elicit a luteinizing hormone (LH) surge. The LH surge triggers ovulation and the luteinization of the ruptured ovarian follicle - critical events for reproduction. We have previously shown that peripheral estradiol (E2) increases neuroprogesterone (neuroP) synthesis in the hypothalamus in postpubertal astrocytes. This newly synthesized neuroP stimulates kisspeptin release to initiate the LH surge. Interestingly, E2-facilitated neuroP synthesis by hypothalamic astrocytes only occurs in post-pubertal females and has not been observed in prepubertal females in vivo, prepubertal hypothalamic astrocytes in vitro or males at any age - including male astrocytes in vitro. How puberty affects hypothalamic astrocytes to begin to allow E2-facilitated neuroP release has not been elucidated. The focus of the current study is to determine if the cellular machinery responsible for neuroP release is upregulated in adult female mice compared to prepubertal females. Of particular interest are translator protein (TSPO) and StAR (steroidogenic acute regulatory protein), which mediate the rate-limiting step in steroidogenesis, and 3beta-hydroxysteroid dehydrogenase (3β-HSD), an enzyme that catalyzes the biosynthesis of progesterone. Hypothalamic astrocyte cultures were generated from female mice at two different developmental ages: prepubertal (postnatal day (P) 23) and adult (~P60). The ability to release neuroP in response to E2 was monitored via progesterone ELISA. Western immunoblotting and quantitative real-time PCR was performed to determine differences in TSPO, StAR, and 3β-HSD protein and RNA, respectively. Determining if there are developmental increases in the expression of the cellular machinery involved in neuroP synthesis will provide a mechanism for the development of E2-facilitated neuroP synthesis in hypothalamic astrocytes.

Disclosures: **M.A. Mohr:** None. **P. Micevych:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Topic: B.12. Glial Mechanisms

Support: Grant-in-aid for Scientific Research on Inno- vative Areas; Grant number: 24116007

Title: Impact of the GFAP gene variant rs2070935 on the structure and function of the human brain

Authors: *Y. TAKAHASHI, M. SAKAI, Z. YU, H. TOMITA;
Dept. of Disaster Psychiatry, Tohoku Univ., Sendai-Shi, Japan

Abstract: Grial fibrillary acidic protein (GFAP) is one of the main intermediate filament proteins in astrocytes. GFAP has key roles in maintainance of mechanical strength of astrocyte, astrocyte-neuron communication during synaptic plasticity, and reactive gliosis. GFAP is widely used as a marker for astrocytes. GFAP is induced upon brain damage or during central nervous system degeneration. Accumulating evidence suggests involvement of GFAP dysregulation in the pathogeneses of various psychiatric or neurological disorders. Expression changes in GFAP have been reported in the brain from patients with schizophrenia, bipolar disorder, major depressive disorder, Alzheimer disease, and Parkinson disease. Also, GFAP mutations cause a rare, progressive and often fatal neurodegenerative disorder, Alexander disease, which is characterized by cytoplasmic inclusion of GFAP in astrocytes. Recently, C allele of a single nucleotide polymorphism (SNP), rs2070935 (A/C), located in GFAP promoter region, has been shown to be related with upregulation of GFAP and severe symptom of alexander disease, which suggests that the polymorphism may affect brain function as well as susceptibility to psychiatric or neurological disorders. However, the associations between the functional SNP and brain structure and function remain unelucidated. To investigate effect of the genetic variations in GFAP on the structure and function of the human brain, we evaluated association between the SNP and MRI findings, including gray and white matter volumes, mean cerebral blood flow during rest using arterial spin labeling, and diffusion tensor imaging(DTI)-based white matter integrity among 1212 healthy Japanese people. The C allele of the SNP was significantly associated with decreased volume of gray and white matter in inferior frontal lobes, decreased cerebral blood flow in broad area in cerebrum, and increased mean diffusivity in subcortical region of left temporal and posterior lobes. These variations in volumes, diffusivities, activities of the brain modulated by the genetic variation in GFAP might be relevant to susceptibility to neuropsychiatric and neurodegenerative disorders.

Disclosures: **Y. Takahashi:** None. **M. Sakai:** None. **Z. Yu:** None. **H. Tomita:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Program#/Poster#: 509.12/R14

Topic: B.12. Glial Mechanisms

Support: BBSRC grant BB/J017809

Title: Astrocyte mediated neuronal synchronisation properties revealed False Gliotransmitter release

Authors: *R. PARRI[1], G. SAUNDERS[1], N. CODADU[2], T. M. PIRTTIMAKI[3], R. E. SIMS[1]; [1]Aston Univ., Birmingham, United Kingdom; [2]Newcastle Univ., Newcastle, United Kingdom; [3]Univ. of eastern Finland, Kuopio, Finland

Abstract: Astrocytes spontaneously release glutamate as a gliotransmitter (GT) resulting in the generation of extrasynaptic NMDA-R mediated slow inward currents (SICs) in neighbouring neurons, which can increase local neuronal excitability. However, there is a deficit in our knowledge of the factors that control spontaneous astrocyte GT release, and the extent of its influence. We found that increasing the supply of the physiological transmitter glutamate increased the frequency (control : 0.066±0.01 /min; Pre-Glut: 1.26±0.2 SICs/min; $p<0.0001$; n=50, 25 cells respectively) and signalling charge of SICs in VB thalamus over an extended period. This phenomenon was replicated by exogenous pre-exposure to the amino acid D-Aspartate (0.74±0.23 SICs/min, n=10 compared to 0.062±0.01 SICs/min,n=15, in control). SIC frequency increase for glutamate and D-Aspartate was abrogated by co-treatment with TBOA indicating a role for EAAT uptake. Using D-Aspartate as a "False" GT we determined the extent of local neuron excitation by GT release in VB thalamus, CA1 hippocampus and somatosensory cortex. By analysing synchronised neuronal NMDA mediated excitation we found that the properties of the excitation was conserved in different brain areas. In the three areas, astrocyte derived GT release synchronised groups of neurons over distances of over 200µm. Individual neurons participated in more than one synchronised population, indicating that individual neurons can be excited by more than one astrocyte, and that individual astrocytes may determine a neuron's synchronised network. The results confirm that astrocytes can act as excitatory nodes that can influence neurons over a significant range in a number of brain regions. Our findings further suggest that chronic elevation of ambient glutamate levels can lead to increased gliotransmitter glutamate release, which may be relevant in some pathological states.

Disclosures: R. Parri: None. **G. Saunders:** None. **N. Codadu:** None. **T.M. Pirttimaki:** None. **R.E. Sims:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 509.13/R15

Topic: B.12. Glial Mechanisms

Title: Astrocytic regulation of synaptic transmission in central medial amygdala

Authors: *M. MARTIN-FERNANDEZ[1], G. MARSICANO[2], A. ARAQUE[1];
[1]Univ. of Minnesota, Minneapolis, MN; [2]Univ. of Bordeaux, NeuroCentre Magendie, Bordeaux, France

Abstract: The central medial amygdala (CeM) plays an important role in the responses to fear stimuli, as it is the major output of the amygdala, projecting to fear effector structures. Astrocytes release gliotransmitters that modulate synaptic transmission; however, how astrocytes modulate synapses that produce a different outcome has not been elucidated. The different amygdala subnuclei were identified in acute slices (350 μM thick) from C57BL/6 and $IP3R2^{-/-}$. by transillumination and the CeM nuclei identification was confirmed by the electrical properties of CeM neurons, which were classified as Low Threshold Bursting (54.3%,), Regular Spiking (28.5%), Late Firing (14.3%), and stuttering neurons (2.9%). Using whole cell electrophysiological recordings and calcium imaging techniques, we investigated the astrocytic responsiveness to the endogenous release of endocannabinoids (eCBs) evoked by neuronal depolarization (ND) of CeM neurons. We monitored the intracellular calcium levels of astrocytes, identified with SR101, using the fluorescent dye Fluo4-AM, and induced eCBs release by ND of the recorded CeM neurons. The ND elevated calcium levels in CeM astrocytes, increasing the calcium event probability. This increase in astrocytic calcium event probability was abolished in the presence of the CB1R antagonist AM251, indicating a CB1R-dependent mechanism. The astrocytic response to eCBs was absent in the $IP_3R2^{-/-}$ mice, in which G protein-mediated calcium elevations are selectively impaired in astrocytes, indicating that astrocytic CB1R activation leads to calcium mobilization from internal stores. We then investigated the consequences of the astrocyte calcium signal on the glutamatergic excitatory and GABAergic inhibitory synaptic transmission in CeM neurons. We recorded excitatory or inhibitory postsynaptic currents (EPSCs or IPSCs) evoked by electrical stimulation with an extracellular electrode located in the Basolateral (BLA) or CentroLateral (CeL) sunbuclei, respectively. EPSCs and IPSCs were isolated in the presence of either, AMPAR and NMDAR antagonists, or $GABA_AR$ and $GABA_BR$ blockers, respectively. We depolarized one neuron to induce eCBs release and recorded CeL-CeM IPSCs or BLA-CeM EPSCs in the other paired recorded neuron in order to identify the astrocytic modulation of CeM synaptic transmission. The probability of release of the recorded EPSCs was decreased, with no change in synaptic potency. The

probability of release of IPSCs recorded was increased, with no change in synaptic potency. These data reveal the involvement of astrocytes in synaptic transmission in the CeM

Disclosures: **M. Martin-Fernandez:** None. **G. Marsicano:** None. **A. Araque:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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KAKENHI 16H01888

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HFSP RGP0036/2014

RIKEN Brain Science Institute

Title: Rapid astrocytic control of neural activity by optogenetic Gq-coupled receptor activation *In vivo*

Authors: *Y. IWAI, K. OZAWA, K. YAHAGI, S. SATO, H. HIRASE;
RIKEN Brain Sci. Inst., Wako-Shi, Japan

Abstract: Astrocytes express a wide variety of Gq-type G protein-coupled receptors (GPCRs) and respond to their agonists with intracellular Ca^{2+} elevations. Recent studies show that Gq-GPCR activation by neuromodulators strongly increases astrocytic Ca^{2+} and represents a major mechanism for astrocytic Ca^{2+} dynamics *in vivo* (Bekar et al., 2008; Takata et al., 2011; Ding et al, 2013; Paukert et al, 2014). However, the functional significance of astrocytic Gq-GPCR signaling in neural activity modulation remains controversial (Agulhon et al, 2010), because pharmacological manipulations of GPCRs affect neuronal GPCRs as well. In order to specifically and dynamically activate astrocytic Gq-GPCR signaling *in vivo*, we have generated transgenic mice in which EYFP-fused OptoA1AR, an optically activatable Gq-GPCR (Airan et al., 2009), is expressed under the control of a BAC-GLT1 promoter. Among the transgenic lines

established, several lines showed selective astrocytic expression, with differences observed in their expression strength and positive astrocytic proportion ranging from ~50% to 90%. In these lines, cortical GFAP expression appeared generally low, suggesting that the expression of OptoA1AR-EYFP does not cause glial inflammation. We next confirmed that astrocytic Ca^{2+} elevation was triggered by brief illumination of blue light in anesthetized conditions. *In vivo* rhod-2 Ca^{2+} imaging of the ~50% positive line revealed that a short blue light pulse (~1 s) induced Ca^{2+} elevations within a few seconds in OptoA1AR-EYFP-positive astrocytes but not in negative astrocytes. The magnitude and frequency of spontaneous astrocytic Ca^{2+} surges were similar in both astrocytic populations, and the magnitude of optically-evoked Ca^{2+} surges was comparable to that of spontaneous Ca^{2+} activities. This optical activation was repeatable with an interval of 1 min, although the response magnitude was decreased from the second stimulus onwards. A non-stimulus period of 3 minutes restored the original response magnitude. Remarkably, stronger optical stimulation could induce astrocytic Ca^{2+} wave propagation, in which Ca^{2+} elevations were observed in OptoA1AR-EYFP-negative astrocytes with a delay of several seconds after the activation of the neighboring OptoA1AR-EYFP-positive astrocytes. Furthermore, some of the surrounding neurons were found to be rapidly activated following the astrocytic optical stimulation. We are currently exploring downstream molecular mechanisms and behavioral impacts induced by the astrocytic GPCR activation.

Disclosures: **Y. Iwai:** None. **K. Ozawa:** None. **K. Yahagi:** None. **S. Sato:** None. **H. Hirase:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Topic: B.12. Glial Mechanisms

Support: Foerster-Bernstein PostDoctoral Fellowship

Holland Trice Scholars Award

Title: A candidate screen to identify novel regulators of astrocyte development and astrocyte-synapse interaction

Authors: *K. T. BALDWIN, J. A. STOGSDILL, C. EROGLU;
Dept. of Cell Biol., Duke Univ., Durham, NC

Abstract: Dysfunctional synaptic connectivity is thought to be the underlying cause of many neurological disorders, including autism, schizophrenia, and various forms of intellectual disability. Astrocytes are morphologically complex cells that structurally and functionally interact with synapses to regulate synapse formation and maturation. Disruption of astrocyte-synapse interactions is a major pathological mechanism seen in many neurological disorders, yet whether astrocyte dysfunction contributes to synaptic pathologies in neurological disorders is largely unknown. Interestingly, a number of disease-linked genes encode cell surface proteins that are highly expressed and significantly enriched in astrocytes. However, the function of these genes in astrocytes, and how disruption of their astrocytic function can contribute to neurological disorders, is not known. We hypothesized that many of these astrocyte-enriched disease-linked genes play critical roles in astrocytes to regulate astrocyte development and astrocyte-synapse interactions, and that disruption of their functions in astrocytes critically contributes to synaptic pathologies associated with neurological disorders. To test this hypothesis, we employed an astrocyte and cortical neuron co-culture system to screen carefully selected candidate genes for their role in astrocyte development and astrocyte-synapse interaction *in vitro*. Using this setup, we found that depletion of select candidate genes significantly impairs the ability of astrocytes to establish a complex morphology. Ongoing studies are focused on determining the *in vivo* astrocytic function of these candidate genes using a concurrent labeling and genetic modification strategy. Collectively, these experiments will provide novel insights into the role of astrocyte dysfunction in neurological disorders, and reveal specific molecular mechanisms that facilitate disease pathogenesis.

Disclosures: K.T. Baldwin: None. **J.A. Stogsdill:** None. **C. Eroglu:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Topic: B.12. Glial Mechanisms

Support: CONACYT 79512

CONACYT 387184

Title: Anandamide decreases [^{3}H] D-aspartate uptake activity in cultured Bergmann glia cells

Authors: M. DE JESÚS SÁNCHEZ[1], E. SUÁREZ POZOS[2], L. C. R. HERNÁNDEZ KELLY[2], *C. J. JUÁREZ-PORTILLA[3], A. ORTEGA[2];

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Abstract: Glutamate is the major excitatory transmitter in the vertebrate brain. It acts through specific membrane receptors present in neurons and glial cells. Bergmann glia completely surrounds glutamatergic synapses and it is thought to be involved in their regulation. The endocannabinoid system integrate a regulatory system present in central and peripheral systems, thus it may be involved in the glutamatergic signaling in the brain, particularly in the cerebellum. In order to gain insight the molecular mechanisms of cannabinoid regulation on glutamate signaling we characterized the effect of the activation of cannabinoid receptors in the glutamate uptake process in the well-known model of chick cerebellum primary cultures of Bergmann glial cells stimulated with the endocannabinoid system agonist anandamide. Our first approach was to establish the expression of endocannabinoid receptors CB1 and CB2 in Bergmann glia. Immunochemistry assays as well as western blots demonstrated the expression of both receptors. When the cultured cells were exposed to anandamide, a time and dose-dependent decrease in [^{3}H]D-Aspartate uptake activity was detected. A detailed kinetic analysis demonstrated that the effect of anandamide is related to a decrease in the plasma membrane glutamate transporters. These results strength the notion of glial cells as targets of endocannabinoids and support the idea of a critical involvement of glia cells in the function and dysfunction of the brain.

Disclosures: M. De Jesús Sánchez: None. **E. Suárez Pozos:** None. **L.C.R. Hernández Kelly:** None. **C.J. Juárez-Portilla:** None. **A. Ortega:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Program#/Poster#: 509.17/S2

Topic: B.12. Glial Mechanisms

Title: Dietary impact on astrocytes in the arcuate nucleus of the hypothalamus

Authors: *J. GAMMONS[1,2], A. SMITH[1], K. O'CONNELL[2,3];
[2]Physiol., [3]Neurosci. Inst., [1]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Obesity leads to chronic metabolic disruption and continues to be a rising epidemic. Centrally projecting neurons from the arcuate nucleus of the hypothalamus orchestrate feeding behavior, and previous work for our lab demonstrates their altered biophysical properties in response to the consumption of high levels of dietary fats, prior to gaining weight. However, it remains unclear to what extent food intake is under the control of glial cells, which are well

known to contribute to inflammatory processes associated with obesity. Astrocytes function to maintain homeostatic conditions for neuronal activity and regulate neurotransmission. To investigate the impact of high-fat diet (HFD) consumption on astrocytic excitability, mice were fed HFD ad libitum for 2 days or 3 weeks. Recordings from acute brain slices using whole-cell patch clamp were used to compare the effects of HFD to chow-fed and fasting animals. Our data suggest that the electrophysiological properties of astrocytes located in the arcuate are feeding-state dependent and are distinctively altered by short-term, as well as chronic, exposure to high fat diet. Using immunohistochemistry, we discovered that expression of astrocytic K_{ir} 4.1 was significantly decreased in the brains of mice fed HFD. To this end, we characterized the effect of diet on Ba^{2+}-sensitive currents in arcuate astrocytes. Additionally, we assessed the integrity of the astrocytic network by measuring gap junction coupling and intracellular Ca^{2+} signaling using a genetically encoded Ca^{2+} indicator. These findings suggest prodromal astrocytic dysfunction may contribute to obesogenic factors that precede weight gain.

Disclosures: **J. Gammons:** None. **A. Smith:** None. **K. O'Connell:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 509.18/S3

Topic: B.12. Glial Mechanisms

Support: NIH P50GM068762

NMSU Manasse Endowment

Title: Autophagy in astrocytoma monolayer and hydrogel cell culture systems.

Authors: ***M. P. JOGALEKAR**[1], L. G. COOPER[2], E. E. SERRANO[2];
[1]Mol. Biol., [2]Biol., New Mexico State Univ., Las Cruces, NM

Abstract: Astrocytoma is a primary malignant brain tumor that invades the surrounding tissue. The cancer is aggressive and the estimated survival rate of patients following diagnosis is 12-15 months. Cell lines derived from human donors diagnosed with astrocytoma are an essential tool for *in vitro* studies of disease progression and treatment. Traditional methods for cell culture maintain cells in monolayer (2D) environments, with growth on a rigid substrate and immersed in liquid media. However, a growing body of evidence suggests that cancer cells maintained in hydrogel environments (three-dimensional; 3D) are biologically more similar to *in vivo* tumor tissue (Kenny et al, 2008; doi: 10.1016/j.molonc.2007.02.004). We undertook the current study

to assess whether a 3D cell culture platform can afford utility in developing interventions against astrocytoma. To this end, the Grade IV astrocytoma cell line ATCC CCF-STTG1 (a glioblastoma) was maintained in conventional monolayer culture on tissue culture polystyrene, and in 3D culture using the extracellular matrix hydrogel, Geltrex^{TM}. When cells were imaged with phase contrast and laser confocal imaging methods, we observed that cells grown on polystyrene appeared flat-layered in sheets, adhered to the chamber surface, and grew parallel to the substrate. In contrast, 3D matrix-organized cells formed compact multilayer aggregates interspersed with regions of cell clusters . Ultrastructural analysis with transmission electron microscopy (TEM) showed a prevalence of autophagic structures in both 2D and 3D cultures, an observation consistent with prior TEM studies from our laboratory with the triple negative breast cancer line HCC70. Analysis of an open source GEO DataSet (GSM886923) uncovered expression of 219 of 222 genes implicated in autophagic pathways by the Human Autophagy Database (HADb). Microarray measurements of gene expression ranged from 3.2 to 14.7 arbitrary units of fluorescence (AU; 8.1 ± 2.4; mean ± S.D.) and included detection of BECN1 (9.3 AU), PIK3 (8.7 AU), and BCL2 (5.1 AU). Our results demonstrate that the organization of astrocytoma cells in culture can be influenced by the surrounding microenvironment. Moreover, ultrastructural and molecular characteristics of autophagy are features of this astrocytoma cell line. Future studies will analyze the molecular pathways for autophagy using fluorescent probes and expression analysis methods with the goal of identifying potential molecular targets for control of astrocytoma cell proliferation.

Disclosures: **M.P. Jogalekar:** None. **L.G. Cooper:** None. **E.E. Serrano:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Program#/Poster#: 509.19/S4

Topic: B.12. Glial Mechanisms

Support: IEF Marie Curie, EPUE009801

Title: Astrocyte regulation of neuron circadian behavior

Authors: ***O. BARCA-MAYO**, M. PONS-ESPINAL, L. BERDONDINI, D. DE PIETRI TONELLI;
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Abstract: Circadian rhythms govern a large variety of physiological and metabolic functions. Although growing evidence suggests a major involvement of astrocytes in the regulation of

circadian rhythms in invertebrates, its role in mammals is currently unknown. Using rodent as main model, we characterize the role of astrocytes as modulators of neuronal rhythms by performing co-culture of synchronous astrocytes with asynchronous neurons onto physically separated layers, but sharing the same culture media. We found that astrocytes induce synchronization of circadian oscillators genes in neurons. Moreover, *Bmal1* knockdown in astrocytes partially suppresses circadian entrainment in cortical neurons, suggesting that astrocytes are competent circadian oscillators able and required to synchronize cortical neurons in vitro. Additionally we generated an inducible conditional mouse model where Bmal1 can be deleted in astrocytes and found impaired oscillation of canonical clock genes in cortex and hippocampus of the mutant as compared to control mice. Mutant mice displayed altered circadian locomotor activity, severe cognitive impairments and peripheral metabolic abnormalities. Those results suggest that astrocyte circadian rhythms interface and modulate the neural circuitry thereby controlling neuronal rhythmic behavior at cellular, tissue and organism level.

Disclosures: O. Barca-Mayo: None. **M. Pons-Espinal:** None. **L. Berdondini:** None. **D. De Pietri Tonelli:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Topic: B.12. Glial Mechanisms

Support: DFG, CRC 1080

ERC-AdG “LiPsyD”

Title: Action of autotaxin (ATX) at the glutamatergic synapse

Authors: *C. THALMAN[1], G. HORTA[1], N. FERREIROS[2], I. TEGEDER[2], S. KIRISCHUK[3], K. RADYUSHKIN[4], J. RÖPER[5], J. VOGT[1], R. NITSCH[1];
[1]Inst. of Microscopic Anat. and Neurobio., Mainz, Germany; [2]Inst. für Klinische Pharmakologie, Frankfurt, Germany; [3]Inst. of Physiol. and Pathophysiology, Mainz, Germany; [4]Mouse Behavior Unit, Mainz, Germany; [5]Inst. for Neurophysiol., Frankfurt, Germany

Abstract: Recently, bioactive lipids, such as lysophosphatidic acid (LPA), were described to be involved in the control of neuronal signaling. We have recently reported on plasticity related gene 1 (PRG-1), a phospholipid interacting molecule, acting as a regulator of LPA signaling at

excitatory synapses. In order to understand the molecular function of LPA, we focused our work on the role of autotaxin (ATX), the main LPA-synthetizing molecule, at glutamatergic synapses. We show that ATX is expressed in the astrocytic compartment of the excitatory tripartite synapse and that its secretion is regulated by glutamate acting on AMPA and NMDA astrocytic receptors. To gain further insight into the effect of the LPA/ATX axis, we performed electrophysiological recordings where ATX activity was either inhibited by pharmacological blockers or genetically deleted in astrocytes. While pharmacological inhibition disrupted ATX function in the neuronal network, genetic deletion allowed us to selectively delete ATX in astrocytes and to inhibit the LPA-synthesis at the synaptic cleft. In fact, inhibition of LPA synthesis was able to normalize excitation/inhibition balance under pathophysiological conditions associated with hyperexcitability. Our data underline the role of synaptic lipid signaling in the regulation of excitatory transmission in the brain.

Disclosures: **C. Thalman:** None. **G. Horta:** None. **N. Ferreiros:** None. **I. Tegeder:** None. **S. Kirischuk:** None. **K. Radyushkin:** None. **J. Röper:** None. **J. Vogt:** None. **R. Nitsch:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 509.21/S6

Topic: B.12. Glial Mechanisms

Title: Fananas cells: the forgotten type of cerebellar astroglia?

Authors: A. GOERTZEN[1], *R. W. VEH[2];
[1]Klinik für Neurologie, Katholisches Klinikum Oberhausen, Oberhausen, Germany;
[2]Universitaetsklinikum Charite, Berlin, Germany

Abstract: For long times astrocytes were regarded as non excitable cells, passively filling the spaces between neuronal cell bodies, dendrites, axons, and synapses. During the last decades, however, it has been recognized that astrocytes are involved in a variety of biological functions such as regulating cerebral blood flow, supporting neuronal metabolism, controlling the extracellular potassium concentration, and clearing neurotransmitters between synapses. In addition to principal forms like protoplasmic and fibrous types, in line with so many biological functions, astrocytes display a conspicuous regional heterogeneity including choroid plexus epithelial cells, marginal glia, tanycytes, radial astrocytes, retinal Müller glia, vellate glia, and cerebellar Bergmann glial cells.
The cerebellum is thought to host several types of astrocytes: protoplasmic, fibrous, vellate and Bergmann cells. At present it is not known, whether these glial cells together are sufficient to

cope with the increasing complexity of cerebellar functions. The older literature presents an additional potentially astrocytic glial type: the Fananas cell, which, if existing in reality, has completely disappeared from scientific awareness. These cells were identified by a special staining procedure (gold sublimate technique) at the beginning of the 20th century. Their structure is similar but not identical to that of Bergmann cells, making the differentiation just by morphological methods impossible. Based on our long standing interest in morphology and function of the cerebellum we set out to use a battery of about 50 antibodies against potassium channels and related proteins to find molecular support for the existence of Fananas cells in the mammalian brain. Indeed we could identify the Kv2.2 potassium channel protein as molecular marker for Fananas cells. This finding may be helpful to characterize potential biological functions of this forgotten glial cell type.

Disclosures: **A. Goertzen:** None. **R.W. Veh:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Topic: B.12. Glial Mechanisms

Support: CRG3 "KAUST-EPFL Alliance for Integrative Modeling of Brain Energy Metabolism"

Title: Detailed morphometric analysis of a glial process in the adult rat hippocampus

Authors: ***C. CALI**[1], K. KARE[2], D. J. BOGES[2], H. LEHVASLAIHO[2], P. J. MAGISTRETTI[2,3]; [2]BESE, [1]KAUST, Thuwal, Saudi Arabia; [3]Brain and Mind Inst., EPFL, Lausanne, Switzerland

Abstract: Early observers including Cajal and Golgi, noted the complex morphology of astrocytes, and showed that they are strategically located at the interface between neurons and vasculature in the brain. Astrocytes have been shown to be involved in a numerous tasks in the CNS that include metabolic support of neuronal function and regulation of chemical compounds in the extracellular space, in order to sustain synaptic transmission. This is also reflected in their complex morphology and intimate relationship with all elements of the neuropil. A detailed knowledge of the morphology of these cells at the resolution that is below that of light microscopy can shed light on astrocytic functions. In particular, perisynaptic processes, that cannot be currently investigated *in vivo* or *in situ*, can be reconstructed in detail using electron microscopy imaging and three-dimensional reconstruction *in silico*. Here, we describe in detail a 220 cubic micron volume of adult rat hippocampus, where we reconstructed all the cellular

components, with particular emphasis on the astrocytic processes. This gave us information on astrocytic coverage, extent of individual synapses, the widespread presence of ER and mitochondria in lamelliform processes, and their proximity to spines, boutons, and PSDs. This information provides the structural basis for understanding higher-level phenomena that involve astrocyte-neuron crosstalk, like ANLS or calcium-induced-calcium-release.

Disclosures: **C. Cali:** None. **K. Kare:** None. **D.J. Boges:** None. **H. Lehvaslaiho:** None. **P.J. Magistretti:** None.

Poster

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Topic: B.12. Glial Mechanisms

Support: NIH Grant R01 MH099555-03

Title: Exploring the maturation of human iPSC-derived astrocytes in 3D cerebral cortical cultures

Authors: ***S. A. SLOAN**[1], S. DARMANIS[2], N. HUBER[3], F. BIREY[3], C. CANEDA[1], S. R. QUAKE[2], B. A. BARRES[1], S. P. PASCA[3];
[1]Neurobio., [2]Bioengineering, [3]Psychiatry, Stanford Univ., Palo Alto, CA

Abstract: Astrocytes play critical roles in nervous system function and contribute to the pathophysiology of multiple neuropsychiatric disorders. Therefore, there is a significant need to develop physiologically relevant cellular models for investigating human astrocytes in both health and disease. Here we present an approach for reliably generating human astrocytes from induced pluripotent stem cells (iPSC) in a three-dimensional (3D) cytoarchitecture using human cerebral cortical spheroids (hCS). By tailoring existing immunopanning techniques designed to harvest astrocytes from primary human samples, we were able to acutely purify astrocytes and neurons from hCS and perform high-depth RNA-sequencing and single cell RNA-sequencing of iPSC-derived astrocytes to directly compare with primary human astrocytes. By leveraging long-term cultures of hCS (over 500 days), we were able to transcriptionally and functionally capture astrocytes at several *in vitro* developmental stages. We found that iPSC-derived astrocytes in hCS closely resemble primary human fetal astrocytes and that over time they transition from a predominantly fetal to a mature human astrocyte signature. Importantly, these changes are accompanied by age-dependent changes in the functional properties of astrocytes. Together, these data suggest that astrocytes generated within a hCS system resemble primary human

astrocytes with high fidelity, and that the intrinsic and/or extrinsic signals required for astrocyte maturation are present in long-term cultures of hCS.

Disclosures: **S.A. Sloan:** None. **S. Darmanis:** None. **N. Huber:** None. **F. Birey:** None. **C. Caneda:** None. **S.R. Quake:** None. **B.A. Barres:** None. **S.P. Pasca:** None.

Poster

509. Astrocyte Cell Biology and Modulation II

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Support: 2014R1A6A1029617

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Title: Astrocytic glucose metabolic flow correlates with high expression of LDH and implicates in radical proliferation ability of GBM.

Authors: ***J. KIM**[1,2], J. HAN[1], Y. JANG[1,2], S. KIM[1,2], M. LEE[1,2], I. RYU[1,2], M. RYU[1], G. KWEON[1,2,3], J. HEO[1,2,4];
[1]Dept. of biochemstry, [2]Dept. of medical science, [3]Res. Inst. for Med. Sci., [4]Brain research institute, Chungnam Natl. Univ. Sch. of Med., Daejeon, Korea, Republic of

Abstract: Among the primary brain tumors, glioblastoma multiforme (GBM) has a radical proliferation ability that complicates the therapeutic modulation of cancer progression. The majority of GBM patients have a low survival rate (<1 year) due to radical tumor growth and late cancer diagnosis. Previous reports have shown that astrocytes have a specific metabolic organization that includes the production of lactate, the storage of glycogen, and use of lactate to support neurons which possess higher capacity of metabolism compared to neurons. We hypothesized that these characteristics of astrocytes could contribute to enhanced proliferation of GBM compared to neuroblastoma (NB). Here, we show that U87MG cells (a model of GBM) proliferate more rapidly than SH-SY5Y cells (a model of NB). A higher extracellular acidification rate and maximal mitochondrial oxygen consumption rate were observed in U87MG cells compared to SH-SY5Y cells. The expression levels of lactate dehydrogenase (LDH)-A and LDH-B were higher in U87MG cells and primary cultured astrocytes than in SH-SY5Y cells and neurons. Furthermore, the mRNA levels of *succinate dehydrogenase* and *peroxisome proliferator-activated receptor-γ* were high in U87MG cells, suggesting that these

cells have high capacity for mitochondrial metabolism and uptake of fatty acids related to synthesis of the cell membrane, respectively. Taken together, we demonstrate that GBM cells are characterized by activation of the LDH-expression-related glycolytic pathway and mitochondrial metabolic capacity, suggesting two innate properties of astrocytes that could provide a driving force for the growth ability of GBM. Based on these findings, we propose that therapeutic approaches aimed at treating GBM could target LDH for modulating the metabolic properties of GBM cells.

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Poster

509. Astrocyte Cell Biology and Modulation II

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Program#/Poster#: 509.25/S10

Topic: B.12. Glial Mechanisms

Support: NIH R01MH099554

Title: Molecular specification of functionally heterogeneous dorsoventral and subcortical astrocyte sub-populations in adult central nervous system (CNS)

Authors: ***Y. YANG**[1], L. MOREL[1], M. CHIANG[1], H. HIGASHIMORI[1], R. BRADLEY[2], L. IYER[1], S.-C. ZHANG[2];
[1]Neurosci., Tufts Univ. Sch. of Med., Boston, MA; [2]Waisman Ctr., Univ. of Wisconsin, Madison, WI

Abstract: *In vivo* molecular markers that characterize heterogeneous astroglial populations in adult CNS remain essentially unknown. In the current study, we profiled ribosome-associated mRNA, presumably actively translating mRNA in astrocytes, from adult (P70) cortical (cortex and hippocampus) and subcortical (caudate putamen, nucleus accumbens, thalamus, and hypothalamus) regions using translational ribosome affinity purification (TRAP) and RNA-Seq techniques. Our results found that the similarity of ribosome-associated mRNA profile in astrocytes closely follows the dorsoventral axis, especially posteriorly from cortex to hypothalamus. We further identified specific mRNA transcripts that showed significantly differential expression patterns in region-specific astrocytes. These genes include modulators of synaptogenesis (*sparc*, *thsb4*), enzymes (*agt*), and transcription factors (*emx2*, *hopx*, and *lhx2*). Their differential expression patterns were confirmed by qRT-PCR and immunostaining in adult mouse CNS. Moreover, the differential expression pattern of identified genes was also validated

in human dorsal or ventral forebrain astrocytes derived from induced pluripotent stem (ips) cells. By establishing region mis-matched neuron and astrocyte co-cultures, we further showed that subcortical astrocytes induce significantly fewer numbers and less mature glutamatergic synapses when compared to cortical astrocytes, consistent with the expression pattern of synaptogenic molecules (*sparc*, *thsb4*) in astrocytes from these regions. By examining astrocyte labeling on EAAT2-tdT^{+} ALDH1L1-eGFP^{+} reporter mice, we next identified tdT^{-}eGFP^{+}, tdT^{low}eGFP^{+}, and tdT^{high}eGFP^{+} cortical astrocyte sub-populations. Interestingly, tdT^{-}eGFP^{+} astrocytes are selectively distributed in cortical layer I-II and exhibit significantly different membrane and potassium channel properties. These sub-populations were subsequently isolated through fluorescence active cell sorting (FACS) and their mRNA profile was compared following RNA sequencing. The tdT^{-}eGFP^{+} astrocytes also showed unique molecular signatures when compared to tdT^{low}eGFP^{+} or tdT^{high}eGFP^{+} cortical astrocytes. In summary, our study characterized molecular signatures of dorsoventral and cortical subpopulation astrocytes, providing new molecular markers to better understand their heterogeneous functions in adult CNS.

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Poster

509. Astrocyte Cell Biology and Modulation II

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Topic: B.12. Glial Mechanisms

Title: Astrocytes control microglia reactivity through TSPO

Authors: *O. CHECHNEVA[1], F. MAYRHOFER[2], W. DENG[3,2];
[1]Biochem. and Mol. Med., Univ. of California, Davis, Sacramento, CA; [2]Shriners Hosp. for Children, Sacramento, CA; [3]Biochem. and Mol. Med., Univ. of California Davis, Sacramento, CA

Abstract: Microglia and astrocytes provide immune surveillance in the central nervous system (CNS) and their intercellular communication is essential for maintaining CNS homeostasis. The mechanisms and molecules involved in astrocyte-microglia cross talk are not well known. Mitochondrial translocator protein (TSPO) is expressed in microglia and astrocytes and its expression is increased during injury to the CNS and used as diagnostic biomarker of neuroinflammation. TSPO is highly expressed in steroidogenic cells and has been implicated in mitochondrial cholesterol import and steroids biosynthesis. Recent findings using conditional

knockout animal models questioned a pivotal role of TSPO in steroidogenesis. The physiological role of TSPO in cellular function is still unclear. We found that knockout of TSPO in postnatal astrocytes *in vivo* using a Cre-LoxP system causes microglia activation and an increased expression of microglia specific calcium-binding protein Iba1 in the brain and spinal cord. Activation of microglia is accompanied by an increased level of pro-inflammatory cytokine IL-1beta mRNA expression. We also observed an increase in TSPO expression in the CNS of knockout animals in association with microglia and blood-brain barrier endothelial cells. Thus, our data showed that loss of TSPO in astrocytes results in activation of microglia and suggests an important role of TSPO specifically in astrocyte-microglia communication and implies the suppression of microglia reactivity by astrocytes for efficient CNS immune regulation.

Disclosures: O. Chechneva: None. **F. Mayrhofer:** None. **W. Deng:** None.

Poster

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Topic: B.12. Glial Mechanisms

Support: NIH Grant R01NS096100-01

Title: Non cell autonomous regulation of proliferation in the injury microenvironment by Sonic hedgehog signaling.

Authors: *R. ALLAHYARI, K. CLARK, B. TING, A. D. R. GARCIA;
Biol., Drexel Univ., Philadelphia, PA

Abstract: Following brain injury, astrocytes undergo a series of biochemical and morphological changes collectively known as reactive astrogliosis. Reactive astrocytes increase expression of glial fibrillary acidic protein (GFAP), undergo proliferation, and participate in complex glial scar formation, segregating the injured from healthy tissue. Understanding the molecular regulators of reactive astrogliosis is key to developing novel therapeutic strategies for treating neurological injury or disease. In this study, we examined the role of Sonic hedgehog (Shh) signaling in reactive astrogliosis. Discrete subpopulations of astrocytes in the adult mammalian forebrain express the transcription factor, Gli1, indicating active Shh signaling. Shh has been shown to play an important role in the neural response to injury. However, the precise role of Shh signaling in regulating reactive astrogliosis is not well understood. We performed forebrain stab injuries in transgenic mice possessing a conditional knock out (CKO) of the obligatory Shh receptor, Smoothened (Smo), specifically in astrocytes (GFAP Smo CKO). Our results show

that injury-induced proliferation is dramatically reduced in GFAP Smo CKO mice. However, the vast majority of proliferating cells at the lesion site do not express Gli1. Moreover, single cell analysis of Gli1-expressing astrocytes at the lesion shows that the majority of Gli1 cells do not undergo proliferation at 3 and 7 days post injury. Consistent with this, glial scar formation is unimpaired in GFAP Smo CKO animals. These data suggest that injury-induced proliferation of Gli1-negative cells is regulated through interactions with Gli1-expressing astrocytes.

Disclosures: R. Allahyari: None. **K. Clark:** None. **B. Ting:** None. **A.D.R. Garcia:** None.

Poster

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NIH-DK-082417

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NIH-NS-060664

NIH-DK-096139

Title: Astrocytes regulate glucagon-like peptide-1 receptor-mediated effects on energy balance

Authors: *D. REINER[1], E. G. MIETLICKI-BAASE[1], L. E. MCGRATH[1], D. J. ZIMMER[1], K. K. BENCE[1], G. L. SOUSA[1], V. R. KONANUR[2], J. KRAWCZYK[1], D. H. BURK[3], S. E. KANOSKI[2], G. E. HERMANN[3], R. C. ROGERS[3], M. R. HAYES[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]USC, Los Angeles, CA; [3]Louisiana State Univ., Baton Rouge, LA

Abstract: The anorectic effects of glucagon-like peptide-1 receptor (GLP-1R) agonists are partly due to direct GLP-1R signaling in the central nervous system (CNS). A small body of literature suggests that GLP-1Rs are expressed on CNS astrocytes. As astrocytes play a critical role in modulating extracellular glutamate, and the hypophagic effects of GLP-1R activation are

partially mediated via glutamatergic signaling, GLP-1R agonists may act directly on astrocytes in feeding-relevant nuclei to regulate energy balance. We hypothesized that GLP-1R ligands act on astrocytes within the nucleus tractus solitarius (NTS), a hindbrain nucleus critical for energy balance control, to affect feeding and body weight in rats. Given the lack of a commercially available GLP-1R-specific antibody, we availed of a novel use of a fluorescently-tagged version of the GLP-1R agonist, exendin-4 (Ex4). Central or peripheral administration of fluorophore-labeled Ex4 localizes within astrocytes and neurons in the NTS, providing evidence of GLP-1R expression in both cell types. Live cell calcium imaging of hindbrain slices showed prolonged Ex4 activation in ~40% of both NTS-astrocytes and neurons. Application of GLP-1R agonists increases cAMP in immortalized astrocytes. These data indicate that astrocytes respond to GLP-1R activation through recruitment of appropriate intracellular signaling cascades. To determine whether endogenous GLP-1 could potentially activate NTS astrocytes, we performed immunohistochemical analyses and show that endogenous NTS-derived GLP-1 axons form close synaptic apposition with NTS astrocytes. Further, inhibition of NTS astrocytes with the astrocyte inhibitor fluorocitrate significantly attenuates the 24h anorectic and body weight-suppressive effects of intra-NTS Ex4, suggesting that NTS astrocytes mediate the energy balance effects of GLP-1R signaling. Preliminary data suggests a down-regulation in gene expression for the astrocytic glutamate transporter 1 (GLT-1) and the glutamate aspartate transporter (GLAST) within the NTS after acute central administration of Ex4, providing a potential cellular mechanism for increased glutamatergic signaling after GLP-1R activation. Collectively, these data demonstrate a role for NTS astrocytic GLP-1R signaling in energy balance control.

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Poster

509. Astrocyte Cell Biology and Modulation II

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2GI2RR00306-26A1

Title: The effect of whisker plucking on the astrocyte-blood vessel interaction in the barrel cortex of neonate and juvenile mice

Authors: *A. MIGIROV, P. SINGH, I. REYES, A. CHAWLA, X. CHEN, L. SHI, A. RODRIGUEZ-CONTRERAS;
Neural Develop., City Col. Ctr. For Discovery and Innovation, New York, NY

Abstract: Astrocytes interact with arteries and microcapillaries through end feet connections that are thought to be involved in neurovascular coupling and the maintenance of the blood brain barrier. Recent studies indicate that manipulations of sensory input affect the development of the vascular network (Lacoste et al., 2014; Whitheus et al., 2014). We hypothesize that depriving sensory input will result in a decreased interaction between astrocytes and blood vessels. To test this hypothesis, we examined the mouse barrel cortex, a model system for studying sensory-dependent plasticity during postnatal development. We used a unilateral whisker-plucking paradigm to reduce activity in the barrel cortex of juvenile mice expressing Cre recombinase driven by the GFAP promoter. The left set of whiskers were plucked daily from postnatal day 15 (P15) until P21, and the right set of whiskers were left intact and used as the control (n=3 mice). At P21, the mice were perfused with fixative solution and their brains were processed with multiple fluorescence labeling using IB4 histochemistry to label blood vessels and anti-GFAP or anti-ALdh1L1 immunohistochemistry to label astrocytes (2-4 slices per mouse). We used confocal imaging and 3D segmentation analysis to visualize and measure vessel and astrocyte labeling volume, and astrocyte-vessel interactions in cortical layer 4 of the barrel cortex. Our preliminary results show that IB4 volume in controls (mean ± sem, in μm^3) was 12526 ± 324 in double-labeled ALdh1L1/IB4 slices and 8532 ± 942 in double-labeled GFAP/IB4 slices. IB4 volume in the whisker pluck condition (mean ± sem, in μm^3) was 12157 ± 344 in double-labeled ALdh1L1/IB4 slices and 8115 ± 990 in double-labeled GFAP/IB4 slices. This is an approximate 3-5% decrease in blood vessel volume in whisker pluck compared to control. However, we did not find apparent changes in astrocyte marker volume. Our next step is to determine if whisker plucking affects astrocyte-vessel interactions during the critical period of vascular development. In current experiments, we are using the same unilateral whisker plucking paradigm, with the difference of whisker-plucking taking place within P0 - P5, the critical period for sensory development and vascular growth in the mouse barrel cortex.

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Poster

509. Astrocyte Cell Biology and Modulation II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 509.30/T1

Topic: B.12. Glial Mechanisms

Support: NIH Grant R01 NS061953

Title: Recombinant adeno-associated virus serotype 6 (AAV6) preferentially transduces astrocytes in the rat brain cortex

Authors: *A. L. SCHOBER[1], D. A. GAGARKIN[1], Y. CHEN[2], L. JACOBSON[1], A. A. MONGIN[1];
[1]Dept. of Neurosci. and Exptl. Therapeut., Albany Med. Col., Albany, NY; [2]ViGene Biosci., Rockville, MD

Abstract: Recombinant AAV vectors are an increasingly popular tool for gene delivery to the CNS, because of their non-pathological nature, low immunogenicity, and ability to stably transduce dividing and non-dividing cells. One of the limitations of AAV vectors is that the majority of them have preferential tropism for neuronal cells. Glial cells, specifically astrocytes, appear to be infected at low rates. Although a number of studies utilized AAV vectors with astrocyte-specific promoters or certain AAV serotypes and pseudotypes with purported selectivity for astrocytes (such as AAVrh43 and AAV2/5), glial infection rates were not consistently high. In the present work, we tested seven common commercially available recombinant AAV serotypes - AAV1, 2, and 5 through 9, all of which expressed GFP under the CMV promoter, for their ability to transduce rodent astrocytes. In primary rat astrocyte cultures, we found that only AAV6 consistently had the highest infection rates. Among the remaining serotypes, AAV2 showed substantial infection rates in some, but not all, of the tested viral batches. As a control, all AAV constructs were re-evaluated in retinal pigmented epithelial cells, which have a high affinity for various AAV serotypes. Based on in the *in vitro* efficacy, we tested cell specificity of AAV6 and AAV2 *in vivo*, which were both injected in the barrel cortex at approximately equal dosages. Three weeks later, brains were sectioned and immunostained for viral GFP and the neuronal marker NeuN or the astrocytic marker GFAP. We found that AAV6 consistently and preferentially transduced astrocytes (95% of cells in the virus-infected areas), but not neurons (13% infection rate). On the contrary, AAV2 infected preferentially neurons (64%), but not astrocytes (20%). Overall, our results suggest that AAV6 can be used as a tool for manipulating gene expression (either delivery or knockdown) in rodent astrocytes *in vivo*. Commercially available AAV6 constructs, packaged on a large scale using standardized techniques, will likely make this approach reproducible across many laboratories.

Disclosures: **A.L. Schober:** None. **D.A. Gagarkin:** None. **Y. Chen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ViGene Biosciences. **L. Jacobson:** None. **A.A. Mongin:** None.

Poster

510. Microglia II

Location: Halls B-H

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Program#/Poster#: 510.01/T2

Topic: B.12. Glial Mechanisms

Support: R21HD075359

R47DA15043

F31 NS078813

F32HL115963-02

5T32MH019938-22

5K08NS075144-05

Title: New tools for studying microglia in the mouse and human CNS

Authors: ***M. L. BENNETT**[1], F. C. BENNETT[2], B. A. BARRES[2];
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Abstract: The specific function of microglia, the tissue resident macrophages of the brain and spinal cord, has been difficult to ascertain because of a lack of tools to distinguish microglia from other immune cells, thereby limiting specific immunostaining, purification, and manipulation. Because of their unique developmental origins and predicted functions, the distinction of microglia from other myeloid cells is critically important for understanding brain development and disease; better tools would greatly facilitate studies of microglia function in the developing, adult, and injured CNS. Here, we identify transmembrane protein 119 (Tmem119), a cell-surface protein of unknown function, as a highly expressed microglia-specific marker in both mouse and human. We developed monoclonal antibodies to its intracellular and extracellular domains that enable the immunostaining of microglia in histological sections in healthy and diseased brains, as well as isolation of pure nonactivated microglia by FACS. Using our antibodies, we provide, to our knowledge, the first RNAseq profiles of highly pure mouse microglia during development and after an immune challenge. We used these to demonstrate that

mouse microglia mature by the second postnatal week and to predict novel microglial functions. Together, we anticipate these resources will be valuable for the future study and understanding of microglia in health and disease.

Disclosures: **M.L. Bennett:** None. **F.C. Bennett:** None. **B.A. Barres:** None.

Poster

510. Microglia II

Location: Halls B-H

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Program#/Poster#: 510.02/T3

Topic: B.12. Glial Mechanisms

Title: Regulation of microglial biology by the chemokine receptor CX3CR1 and brain microenvironment: a RNASeq-based transcriptome analysis using low RNA inputs

Authors: ***S. GYONEVA**[1], R. HOSUR[2], B. COTLEUR[1], K. MIAO[1], N. ALLAIRE[2], C. ROBERTS[2], R. M. RANSOHOFF[1];
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Abstract: Microglia, the brain's resident tissue macrophages, are intimately involved in brain development and proper function into adulthood. Recent evidence suggests that microglial gene expression and functions are shaped by their location across different brain regions. One of the main genetic regulators of microglia is the chemokine receptor CX3CR1. However, the molecular mechanisms underlying CX3CR1 function in microglia are not well understood. Here, we wanted to characterize the role of CX3CR1 on microglial function at the molecular level by examining how deletion of the receptor affects gene expression and thereby cell function. We also examined if *Cx3cr1* deletion affects microglia differentially in the different microenvironment of gray matter, white matter and cerebellum. We isolated microglia from 2 month old $Cx3cr1^{+/+}$, $Cx3cr1^{GFP/+}$ and $CX3CR1^{GFP/GFP}$ mice (GFP disrupting one or both copies of Cx3cr1) using fluorescence-activated cell sorting, which was immediately followed by RNA isolation. For some experiments, the white matter and cerebellum were microdissected from the remaining gray matter from thick brain sections. For transcriptome analysis, we constructed RNASeq libraries using low-input protocols (SMART-Seq v4 technology) with either 1 or 10 ng of total RNA. *Cx3cr1* deletion resulted in the downregulation of many genes involved in immunity response to pathogens, and chemotaxis. The genes upregulated by *Cx3cr1* deletion are generally associated with chromatin binding and transcription. In contrast to *Cx3cr1* genotype, the brain microenvironment had only a modest effect on global gene expression. Our results raise questions about how *Cx3cr1*-knockout microglia may respond to inflammatory challenges and in neurological conditions with inflammatory component.

Disclosures: **S. Gyoneva:** A. Employment/Salary (full or part-time): Biogen. **R. Hosur:** A. Employment/Salary (full or part-time): Biogen. **B. Cotleur:** A. Employment/Salary (full or part-time): Biogen. **K. Miao:** A. Employment/Salary (full or part-time): Biogen. **N. Allaire:** A. Employment/Salary (full or part-time): Biogen. **C. Roberts:** A. Employment/Salary (full or part-time): Biogen. **R.M. Ransohoff:** A. Employment/Salary (full or part-time): Biogen.

Poster

510. Microglia II

Location: Halls B-H

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Topic: B.12. Glial Mechanisms

Support: NIRG-14-321390

ADHS14-080000

Title: A paradigm shift in microglial expression profiles in human brain.

Authors: *D. F. MASTROENI;
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Abstract: Introduction: Conventional wisdom holds that brain microglia are similar regardless of brain region. Array expression data from many labs, including our own show large changes in expression of many glial-specific genes in Alzheimer's disease (AD) compared to normal controls (NC). The problem is however; homogenates are often used to obtain these data which introduces un-wanted complication because of the number of cell types analyzed. Although selected glial-specific changes are distinguishable, there are thousands of genes that are not cell class-specific but play major roles in cell function.**Methods:** In order to investigate disease and regional effects on gene expression we isolated microglial cells by laser captured micro-dissection from CA1 of hippocampus and substantia nigra (SN) of AD, NC and Parkinson's disease (PD) cases, followed by RNA sequencing.**Results:** Laser captured cells allowed more precise definition of relationships between microglia and their expression profiles based on disease and location. In AD CA1 366 significant ($p<.01$) differentially expressed transcripts were observed and 409 in PD CA1. Of those genes which were differentially expressed, less than 5% overlap was observed between AD and PD; implying that different neurodegenerative diseases affect microglia differently in the same brain region. Expanding the analysis to brain regions (e.g. CA1 vs SN) we show over two-thousand differentially expressed genes. These data show that the expression profile within microglial sub-populations are the equally significant among brain regions.**Discussion:** It has been known for more than a decade that microglia have the

ability to release neurotoxic inflammatory factors. These pro-inflammatory factors or cytokines, have prompted hundreds of studies and clinical trials to suppress their function, but none have been successful to date. Although there are several explanations listed in the literature on why these clinical studies failed to recapitulate *in vitro* findings, we hypothesize that these studies failed due to the inability to address probable heterogeneity among glial cells as a function of brain region. These findings lay the foundation for future development of therapeutic targets, aid in the pursuit of new research leads, and answer fundamental biological questions regarding the interplay between glial types and their function based on location, and disease.

Disclosures: D.F. Mastroeni: None.

Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: Medical Research Council MR/K022687/1

University of Southampton Vice Chancellor Studentship

Title: The dynamics of the microglial population in the murine adult brain.

Authors: *K. ASKEW[1], K. LI[3], A. OLMOS-ALONSO[1], F. GARCÍA-MORENO[4], Y. LIANG[3], P. RICHARDSON[1], T. TIPTON[2], K. RIECKEN[5], Z. MOLNÁR[4], M. S. CRAGG[2], O. GARASCHUK[3], V. PERRY[1], D. GOMEZ-NICOLA[1];
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Abstract: Microglia, the brain's resident immune cells, have many functions ranging from control of inflammation in brain disease to monitoring synaptic activity. It has been suggested that the adult microglial population is long-lived and maintained by self-renewal; however the precise mechanisms regulating the temporal and spatial control of this population are unknown. To define the mechanisms we studied the density of the microglial population in the healthy postnatal (P0-P21), adult (4-6 months) and ageing (18-24 months) brain. Complementary techniques were used, including intra-liver labelling of embryonic haematopoiesis to analyse the contribution of infiltrating monocytes to the brain during the perinatal period, pulse-chase

experiments to define the time course of microglial proliferation, long-term *in vivo* imaging of microglia and cell-specific apoptosis-deficient mouse models in order to further analyse proliferation and apoptosis. We found circulating monocytes infiltrate the brain during perinatal stages however these do not contribute to the adult microglial population, which is composed exclusively from yolk sac-derived cells. Our data indicates that the adult cell population displays a far more rapid proliferation rate than previously indicated, accounting for at least 6 renewals of the entire population during the lifetime of the mouse. Activation of the colony-stimulating factor-1 receptor (CSF1R) is largely responsible for controlling microglial turnover under homeostatic conditions. We demonstrate that the number of microglia remains relatively constant from early postnatal stages to aged adulthood due to a balance of proliferation and apoptosis of resident cells. These processes are temporally and spatially coupled, evidenced by synchronicity between proliferative events and cell death observed during long-term *in vivo* imaging. The microglial population undergoes a constant and rapid remodelling, with cell density maintained by a subtle balance of proliferation and apoptosis. This challenges the established view of microglia as long-lived cells to a more dynamic scenario, opening new avenues into the understanding of their roles in the maintenance of brain homeostasis.

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Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: CIHR Grant MOP259183

Dalhousie Medical Research Foundation

Title: Microglia responses to chronic sleep restriction in the rat brain

Authors: *S. HALL[1], S. DEURVEILHER[1], K. SEMBA[1,2,3];
[1]Med. Neurosci., [2]Psychology & Neurosci., [3]Psychiatry, Dalhousie Univ., Halifax, NS, Canada

Abstract: Chronic sleep restriction (CSR) has significant consequences on health and cognition, but underlying mechanisms are unclear. CSR is known to elevate brain levels of inflammatory molecules, and microglia, the resident immune cells of the brain, may be involved in this

neuroinflammatory process. In the current study, we examined microglial responses to CSR using immunohistochemistry for several microglial markers. Rats were sleep restricted using motorized activity wheels to impose cycles of 3 h of sleep deprivation and 1 h of sleep opportunity ('3/1' protocol). We previously showed that this protocol over 4 days induced homeostatic and adaptive changes in sleep measures, sustained attention task performance, and brain-derived neurotrophic factor levels. Adult male Wistar rats were assigned to 8 groups (n = 4-9/group): 4 sleep restricted groups underwent the 3/1 protocol for 3 h, 27 h, 99 h, or 99 h followed by 6 days of recovery, and 4 time-matched locked wheel control groups were kept in stationary wheels. Immediately following respective protocols, rats were perfused for immunohistochemistry using antibodies to microglial markers, including ionized calcium binding adaptor molecule-1 (Iba1), with a standard ABC/DAB-Ni method or fluorescent labels. Significant effects of CSR were seen in 4 of 10 limbic and sleep/wake regions examined. Compared to control levels, both the number of Iba1+ cells and the density of Iba1 immunoreactivity were increased in the prelimbic cortex (PrL) after 27 h and 99 h of CSR, and in the perifornical lateral hypothalamic area, central amygdala (CeA), and dorsal raphe nucleus after 99 h of CSR. After 6 days of recovery following 99 h of CSR, the number of Iba1+ cells was at the control levels in all 4 regions, while the density of Iba1 immunoreactivity remained elevated in all 4 regions except the CeA. Sholl analyses of the morphology of Iba1+ microglia in the PrL revealed greater ramification in layer I than in layer II/III, but no significant effect of CSR. The immune phenotype of microglia was assessed in animals that underwent 99 h of the 3/1 protocol (n=2) and a time matched control group (n=2) using antibodies to OX-6, CD68 (both pro-inflammatory) and arginase 1 (anti-inflammatory). Preliminary analyses showed no significant effect of CSR on the expression of these markers in any of the brain regions studied. These results suggest that microglia respond to the 3/1 CSR protocol and these microglial responses might contribute to the cognitive and health impairments associated with CSR. We are currently investigating whether the region-specific increases in the number of Iba1+ microglia are due to cell proliferation or other mechanisms.

Disclosures: **S. Hall:** None. **S. Deurveilher:** None. **K. Semba:** None.

Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: UK MS Society

Title: Changes in microglia extracellular matrix contribute to age-associated decline in CNS remyelination

Authors: *R. BAROR, R. J. M. FRANKLIN;
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Abstract: Microglia are the immune cells that reside within the central nervous system (CNS) and play important roles in both the healthy and diseased brain. In normal conditions microglia survey their environment in search for new threats, as well as play a role in maintaining synapses homeostasis. During pathological conditions such as demyelination (loss of myelin sheaths) microglia become activated and have an important role in clearing debris, thus contributing to a pro-regenerative environment which can promote remyelination (regeneration of myelin sheaths by newly formed oligodendrocytes). Microglia undergo changes with ageing which render them less effective, specifically in the context of regeneration. In the current study, we have isolated young and aged rat microglia and have discovered that aged microglia increase the production of various proteoglycans. Specifically, we have found that aged microglia increase the expression of NG2 and fibronectin, which are known to inhibit remyelination in the CNS. Our *in vitro* studies show that plating oligodendrocytes progenitor cells (OPC) on de-cellularised aged microglia result in a decrease in the number of cells expressing MBP, a marker of mature oligodendrocytes (OL), suggesting an inhibition of OPC differentiation. Moreover, we have found that these OPC have assumed a different cell fate and mainly differentiated into GFAP+ astrocytes. TGFb levels increase in aged rats serum, and since microglia highly express TGFb receptor, we assessed whether this cytokine can be a potential mechanism for the changes observed in aged microglia. Using qRT-PCR and immunohistochemistry, we have shown that TGFb treated neonatal microglia enhances their expression of NG2 and fibronectin. Furthermore, plating OPC on TGFb-treated neonatal microglia, inhibits their differentiation supporting previously seen effects of aged microglia. In summary, we show that ageing microglia acquire an extracellular phenotype which is inhibitory for OPC differentiation and that potentially contributes to the age-associated decline in remyelination efficiency.

Disclosures: **R. Baror:** None. **R.J.M. Franklin:** None.

Poster

510. Microglia II

Location: Halls B-H

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Program#/Poster#: 510.07/T8

Topic: B.12. Glial Mechanisms

Title: Beyond sex differences: microglial expression of CD36 depends upon estrous stage

Authors: *Y. Y. GRINBERG, C. R. JONAK, R. DILL, D. COSS, M. J. CARSON;
Univ. of California Riverside, Riverside, CA

Abstract: The hippocampus and caudate putamen are two brain structures that are differentially engaged across the estrous cycle of female rodents. Microglia, the brain resident immune cells, are highly plastic cells that influence and respond to physiological changes in neuronal activity. Here we asked whether microglia change in their surveillance and activation state across the estrous cycle. To answer this, we used flow cytometric analysis as a sensitive means to measure cell surface expression of proteins that are markers of microglial activation, in young adult male and female mice. When looking at CD11b, CD45, and Fc receptor (FcR), we found that microglia were largely similar in these healthy unmanipulated animals. However, we did observe modest but significant regional differences in microglial expression of all three molecules, with CD11b and FcR expressed higher in the caudate putamen and CD45 more highly expressed in hippocampus. Further, we observed modest sexually dimorphic expression of CD45 and CD11b, with both higher in females. CD36 is a scavenger receptor that binds to a variety of endogenous and pathogenic ligands. We observed significantly more CD36-positive microglia in the caudate putamen than in hippocampus in both sexes. However, when females were divided into stages of the estrous cycle, this regional heterogeneity in microglial expression of CD36 was lost in the diestrus stage. Thus, CD36 expression was sexually dimorphic in the diestrus stage of the estrous cycle. A cycling of CD36 signaling capacity in the female hippocampus may result in a changing microglial capacity for phagocytosis and initiation of sterile inflammation -processes which can be either neuroprotective or neurotoxic, depending on context.

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Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Title: RNA-seq characterization of mouse and human primary microglia

Authors: *Y. HE[1], X. YAO[2], N. TAYLOR[1], T. LOVENBERG[1], A. BHATTACHARYA[1];
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Abstract: Microglia, the principal immune cells of the brain, play key roles in neuro-glia interaction, neuroinflammation, neural repair, and neurotoxicity. As such, brain microglia have been an intense area of research, both in academia and industry. To understand the role of these cells (morphology, transcriptome, proteome, secretome) in physiology, and perhaps more importantly in the context of a CNS disease, it is critical to establish microglial cell cultures, both from rodents and humans, that represents physiology in a dish. Currently, there are various protocols for primary mouse microglial isolation, including shaking, mild-trypsin digestion, and CD11b magnetic-associated cell sorting (MACS) without head to head comparison of genomic signatures. In this study, we compared the three isolation methods by using postnatal (P0-P3) mouse brains and applied RNA-sequencing technology to determine transcriptomes of isolated murine microglia. Using bioinformatics platform, we compared the transcriptome from the murine microglia to publicly available data from microglia challenged with LPS and Abeta, to provide insight into the degree of spontaneous activation as a result of differences in isolation methodologies. The data demonstrated that microglia isolated by CD11b MACS exhibited the lowest expression level of the 'activation genes', while the other two methods showed higher levels, thereby implying a resting baseline in mouse microglia from CD11b MACS isolation, which can be potentially used for accurate analysis and comparison of gene expression and function in disease models and testing of compounds. To explore whether these findings could be translated to human microglia, we also performed RNA-sequencing to characterize commercially available human microglia. Surprisingly, a human microglia cell line (SV40) (Applied Biological Materials Inc; catalog # T0251) did not show abundant expression of key microglial markers as seen in human primary microglia from ScienCell (catalog #1900) or Clonexpress (catalog # HMG030). In conclusion, we RNA-sequenced the three human microglia cell types, and compared human and murine transcriptomics.

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Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: ANR-10-IAIHU-06

Title: Simplified procedure for producing large yields of functional microglial cells in culture: application for studying neuroinflammatory responses

Authors: *J. E. SEPULVEDA DIAZ[1], M. O. OUIDJA[2], S. B. SOCIAS[3], S. HAMADAT[1], S. GUERREIRO[1], R. RAISMAN-VOZARI[1], P. P. MICHEL[1];
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Abstract: Purified microglial cells in culture are frequently used to model brain inflammatory responses but obtaining large yields of these cells on a routine basis can be quite challenging. Here, we demonstrate that it is possible to achieve high-yield isolation of pure microglial cells from post-natal brain tissue through a simple culture procedure that mainly relies on the adhesion preference of these cells to the polycation polyethyleneimine (PEI) in serum-supplemented DMEM medium. The effects of PEI were not reproduced with other synthetic or biological substrates and they were context-dependent as replacement of DMEM by DMEM/F12 nutrient mixture did not permit microglial cell isolation onto PEI coating. Noticeably, the absence of culture medium renewal during the progression of microglial cell isolation strongly improved cell yield, suggesting that nutritional deprivation was required to make this process as efficient as possible. PEI-isolated microglial cells expressed the macrophage antigen-1 (MAC-1) receptor and also the Fc receptor-like S (Fcrls), a biomarker that specifically distinguishes these cells from other myeloid subsets. When generated in large culture flasks coated with PEI, cultures of microglial cells were easily recovered by trypsin proteolysis to produce subcultures for functional studies. These cultures responded to lipopolysaccharide (LPS, 1-10 ng/mL) treatment by secreting proinflammatory cytokines such as TNF-alpha, IL-6, IL-1beta and by generating nitric oxide and reactive oxygen species. Most interestingly, this response was curtailed by appropriate reference drugs. Microglial cells were also strongly responsive to the mitogenic cytokine GM-CSF, which confirms that the functional repertoire of these cells was well preserved. Because of its high yield and simplicity, we believe that the present method will prove to be especially convenient for mechanistic studies or screening assays.

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Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: NIH grant AG034103 (CJP)

Title: TSPO ligand regulates activation state of cultured glia

Authors: *M. F. UCHOA, S.-J. KIM, P. COHEN, C. J. PIKE;
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Abstract: Neuroinflammation contributes to the pathogenesis of many neurodegenerative diseases, including Alzheimer's disease. One potential strategy to reduce neuroinflammation is activating translocator protein (TSPO). TSPO is an 18kDa protein located mainly in the outer membrane of the mitochondria. In the central nervous system, it is localized mostly in glial cells. TSPO expression is significantly increased in glial cells during chronic neuroinflammation. In fact, TSPO ligands are increasingly utilized as neuroimaging agents for inflammation detection. Interestingly, administration of TSPO ligands to animals decreases inflammatory response, which reveals a potential usage of TSPO ligands as anti-inflammatory therapy. How TSPO activation regulates the various aspects of glial activation is unclear. Based on prior work with immortalized cell line and primary microglia cultures, TSPO ligands have been hypothesized to modulate microglial activity both by reducing pro-inflammatory cytokine production and promoting phagocytic activity. How these actions may be affected by interactions with astrocytes has not been evaluated. To begin addressing this issue, we investigated the effects of TSPO ligands on microglia activation in the presence of astrocytes. Primary mixed glial cultures generated from neonatal rats were treated with vehicle or the classic TSPO ligand Ro5-4864 in the presence or absence of lipopolysaccharide (LPS), a bacterial wall endotoxin that is widely used as an inducer of microglial activation. Cultures were assessed for four established indices of activation: (i) expression of inflammatory cytokines, (ii) morphological evidence of activation, (iii) phagocytosis, and (iv) proliferation. Overall, we observed that LPS significantly increased measures of microglial activation, effects that generally were reduced by Ro5-4864 in a dose-dependent manner. Interestingly, although TSPO ligands have been reported to increase phagocytosis in isolated microglial cells and immortalized cell lines, we observed that Ro5-4864 decreased phagocytosis in microglia cultured in the presence of astrocytes. Collectively, these data indicate that activation of TSPO largely results in decreased activation of microglia in a model that better mimics the brain environment. These findings support the continued evaluation of TSPO ligands as an intervention against microglial-mediated neuroinflammation, a key component of pathogenesis in Alzheimer's disease and numerous other disorders.

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Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: CIHR MOP119578

TGTWF 579063240733

Title: Microglial activation: a tale of two rodent species

Authors: *D. LAM[1,2], S. LIVELY[1], L. C. SCHLICHTER[1,2];
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Abstract: Microglia, the resident immune cells of the CNS, help maintain homeostasis in the healthy brain, but also respond rapidly to perturbations. After an insult, cells release Damage-Associated Molecular Pattern (DAMP) molecules and soluble mediators (e.g., cytokines, free radicals). The resulting microglial activation evokes a complex reactive phenotype that depends on the severity of damage and can evolve over time. The therapeutic goal should be to maximize the beneficial roles of microglia, while dampening their harmful effects, but not nearly enough is known about the spectrum of microglial activation. *In vitro* studies have largely used a simplified classification scheme based on macrophage responses, with two extremes being a pro-inflammatory or 'classical' (M1) phenotype, which is thought to exacerbate tissue damage, and multiple anti-inflammatory (M2) phenotypes. The best characterized M2 states are 'alternative activation' (M2a) and 'acquired deactivation' (M2c), which are thought to mediate tissue repair and inflammation resolution, respectively. While *in vivo* rodent models are being hotly pursued in an effort to translate potential therapies, we do not know whether rats and mice show the same microglial activation responses. The objective of this comparative study of rodent microglia is to quantify molecular responses and several functional consequences of M1 and M2 stimulation, and address the potential to target two K^+ channels to control their activation. Primary microglia from Sprague Dawley rats and C57BL/6 mice were treated with interferon-γ + tumor necrosis factor-α (I+T) to induce a pro-inflammatory state, or with interleukin (IL)-4 or IL-10 to promote two anti-inflammatory states. After 24 hr, we assessed cell morphology, the expression profile of numerous pro- and anti-inflammatory mediators, expression and activity of two K^+ channels (Kir2.1, Kv1.3), and whether these channels regulate migration, proliferation, and nitric oxide (NO) production under the different activation paradigms. Some responses were similar for rat and mouse microglia; e.g., I+T induced NO production and reduced their migration, while IL-4 and IL-10 increased their migration. However, several responses differed; i.e., morphological changes, inflammatory expression profiles, K^+ channel expression and currents. We conclude that the rodent species can affect the outcome of microglial activation and should be further compared in pre-clinical *in vivo* studies.

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Poster

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Heart and Stroke Foundation of Canada

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Title: Complex molecular and functional outcomes of single versus sequential cytokine stimulation of rat microglia

Authors: T. A. SIDDIQUI, *L. C. SCHLICHTER, S. LIVELY;
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Abstract: Background. Microglia are the 'professional' phagocytes of the CNS. Phagocytosis is crucial for normal CNS development and maintenance, but can be either beneficial or detrimental after injury or disease. For instance, white matter damage releases myelin debris that must be cleared by microglia in order for re-myelination to occur but phagocytosis can produce damaging reactive oxygen species. Microglia can acquire pro-inflammatory (M1) or anti-inflammatory (M2) activation states but little is known about the consequences for myelin phagocytosis or the effects of a changing cytokine environment that can occur after injury or disease. **Objective.** The objective was to apply several microglial activation paradigms, with or without myelin debris, and assess: (i) gene expression changes reflecting microglial activation and inflammatory states, and receptors and enzymes related to phagocytosis and ROS production; (ii) myelin phagocytosis and production of reactive oxygen species (ROS); and (iii) expression and contributions of several ion channels that are considered potential targets for regulating microglial behavior. **Methods.** Primary rat microglia were exposed to cytokines, individually or sequentially. First, responses to individual M1 or M2 stimuli were compared: IFN-γ plus TNF-α ('I+T'; M1 activation), interleukin-4 (IL-4; M2a/alternative activation), interleukin-10 (M2c/acquired deactivation). Second, sequential cytokine addition was used to assess microglia repolarization and cell functions. The paradigms were: M2a→M1, M2c→M1, M1→M2a, and M1→M2c. **Results.** M1 stimulation increased pro-inflammatory genes, phagocytosis, ROS, as well as expression of Kv1.3, KCa3.1 and Kir2.1 channels. M2a stimulation increased anti-inflammatory genes, ROS production, and Kv1.3 and KCa3.1 expression. Myelin phagocytosis enhanced the M1 profile and dampened the M2a profile, and both phagocytosis and ROS production were dependent on NOX enzymes, Kir2.1 and CRAC channels. Importantly, microglia showed some capacity for re-polarization between M1 and M2a

states, based on gene expression changes, myelin phagocytosis, and ROS production.
Conclusions. In response to polarizing and re-polarizing cytokine treatments, microglial display complex changes in gene transcription profiles, phagocytic capacity, NOX-mediated ROS production, and in ion channels involved in microglial activation. Because these changes might affect microglial-mediated CNS inflammation, they should be considered in future experimental, pre-clinical studies.

Disclosures: T.A. Siddiqui: None. **L.C. Schlichter:** None. **S. Lively:** None.

Poster

510. Microglia II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 510.13/T14

Topic: B.12. Glial Mechanisms

Support: NHMRC APP1007757

Title: Interferon regulatory factor 8 is a major intrinsic determinant of the microglial cell response to sterile nerve injury

Authors: *I. L. CAMPBELL[1], R. D. XIE[2], N. VILLACAMPA[3], B. ALMOLDA[3], B. GONZALEZ[3], B. CASTELLANO[3];
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Abstract: Interferon regulatory factor (IRF) 8 is an important transcriptional regulator present in many hematopoietic cells including microglia, the resident immune cells of the central nervous system (CNS). We have shown previously(1) that in the normal CNS the properties of microglia are dramatically altered in the absence of IRF8. Here we addressed the question as to whether IRF8 regulates the microglial cell response to sterile nerve injury. Facial nerve axotomy (FNA) was performed in wild type (WT) and IRF8$^{-/-}$ (KO) mice and the brain removed at different days post-injury (dpi). In brains from control IRF8 KO mice, lectin and nucleoside diphosphatase (NDPase) histochemistry revealed gross alterations in the morphology of microglia, which were stunted and hypertrophied but were present in comparable numbers with WT. Following FNA in WT mice, a progressive increase in microglial cell activation was observed peaking at 7 dpi and was accompanied by dense staining for lectin, NDPase and CD11b. By contrast, in IRF8 KO mice, the microglial cell response to FNA was grossly attenuated with the density of staining for lectin, NDPase and CD11b reduced significantly. This diminished microglial cell activation in IRF8 KO mice was paralleled by a significant decrease in microglial cell proliferation at 7 and

14 dpi. The wrapping of individual motor neuron cell bodies in the axotomised facial nucleus by microglia involved in synaptic stripping and phagocytosis was incomplete in IRF8 KO mice and was accompanied by an increase in degenerated neurons at 21 dpi. These findings show that in addition to maintenance of homeostatic properties, IRF8 also has an important role in regulating a number of key processes in the microglial cell response to neuronal injury. [(1)]Minten, C. et al. PLoSOne. 7(11): e49851

Disclosures: I.L. Campbell: None. **R.D. Xie:** None. **N. Villacampa:** None. **B. Almolda:** None. **B. Gonzalez:** None. **B. Castellano:** None.

Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: NIH Grant R15NS095314

NSF Grant DBI-0821211

Academic Challenge Grant from the Department of Biology

Title: Investigating the role of microglia in the spinal cord plasticity observed following peripheral axon injury

Authors: *J. MALONEY, L. G. ISAACSON;
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Abstract: Our lab has previously reported that transection of distal axons in the cervical sympathetic trunk (CST) leads to robust retrograde neuronal and glial plasticity in the upper thoracic spinal cord. At one week following injury we observed a marked transient plasticity in the discrete population of parent sympathetic preganglionic neurons housed in the intermediolateral cell column (IML). This plasticity included a decrease in cell body volume, decreased expression of choline acetyltransferase (ChAT), and exclusive expression of activating transcription factor 3 (ATF3) by injured neurons. In addition to neuronal plasticity in the IML, an increase in the number of activated microglia, characterized by aggregation, amoeboid morphology and retraction of cell processes along with increased expression of the pro-inflammatory cytokine interleukin 1β (IL-1β) were observed following peripheral axon injury. Because pro-inflammatory cytokines secreted by activated microglia have been shown to signal to surrounding cells, we hypothesized that microglial activation following injury contributes to

the observed neuronal plasticity. The objective of the present study was to assess the effects of minocycline, a tetracycline-class antibiotic, on microglia activation in the upper thoracic spinal cord following CST transection for use as a model to determine whether activated microglia influence neuronal plasticity. Starting on the day of injury, rats received daily intraperitoneal injections of minocycline (50mg/kg for two days, 25mg/kg for five days; n=6) or vehicle (VEH; saline; 2ml/kg; n=6) for one week. At one week following injury, we found no reduction in the number of microglia cells expressing ionized calcium-binding adapter molecule 1 (Iba1) in the IML. However, the microglia exhibited fewer obvious morphological signs of activation. A blinded quantitative analysis of microglial activation based on cellular morphology revealed that microglia activation in minocycline-treated animals was significantly reduced by 58%. In addition the increased expression of IL-1β typically observed at one week following injury was significantly reduced by 37% in minocycline-treated animals. We conclude that minocycline administration reduces both the extent of microglia activation and the expression of pro-inflammatory cytokines in the vicinity of the injured preganglionic cell bodies in the IML following peripheral axon injury. These results lay the groundwork for future studies examining the role of microglia in the neuronal plasticity observed following injury.

Disclosures: J. Maloney: None. **L.G. Isaacson:** None.

Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

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NIH R21DE025689 to L-J. W.

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New Jersey Commission on Spinal Cord Research CSCR15ERG015 to L-J. W.

Title: Microglia and monocytes synergistically promote the transition from acute to chronic pain after peripheral nerve injury

Authors: *J. PENG[1,2], N. GU[1], L. ZHOU[1], U. B. EYO[1], M. MURUGAN[1], W.-B. GAN[2], L.-J. WU[1];

[1]Cell biology and Neurosci., Rutgers Universiey, Piscataway, NJ; [2]Dept. of Neurosci. and Physiol., New York Univ. Sch. of Med., New York, NY

Abstract: After peripheral nerve injury, spinal microglia and peripheral monocytes participate in neuropathic pain hypersensitivities. However, the respective function of microglia and peripheral monocytes has not been investigated. Here, using $CX_3CR1^{CreER/+}$:$R26^{iDTR/+}$ mice, in which we can specifically and temporally control the depletion of microglia/monocytes, we examined the role of microglia/monocytes in the initiation and maintenance of neuropathic pain in a mouse model of spinal nerve transection (SNT). Tamoxifen (TM, 150 mg/kg, i.p., 4 injections with 48 hr intervals) was used to induce Diphtheria Toxin (DT) receptor expression in Cx_3CR1^+ cells. In 3 days after the last TM injection, DT (i.p. 50 μg/kg, 2 injections with 48 hr interval) treatment can reliably ablate both central microglia and peripheral CX_3CR1^+ monocytes. We found that, with DT treated 24 hr before and 24 hr after SNT surgery, the neuropathic pain development was fully blocked; with DT treated at Post Operation Day (POD) 3 and POD5, when the neuropathic pain has initiated, the pain hypersensitivities were partially reversed to till at least testing period of 14 days; however, with DT treated at POD7 and POD9, the pain hypersensitivities was only transiently reversed. Therefore, we pinpointed a critical time window that requires microglia/monocytes during the initiation of neuropathic pain. The peripheral blood monocytes have substantially rapid turnover and are replenished frequently. The new generated $CX3CR1^+$ monocytes will not express DT receptor without TM treatment since the progenitor cells do not express CX_3CR1. Therefore, we can ablate the central microglia with DT but leave the peripheral blood monocytes intact at 3 weeks after the TM injection. With this method, we found that the ablation of central microglia can only delay but not prevent the development of SNT-induced neuropathic pain. We then used liposome-encapsulated clodronate to deplete peripheral monocytes, which itself did not affect the neuropathic pain development. However, the combination of clodronate with central microglia ablation was able to completely reverse SNT-induced neuropathic pain hypersensitivities. The results demonstrated that both resident microglia and peripheral monocytes are critical in gating neuropathic pain after SNT. We propose that resident microglia and peripheral monocytes synergistically initiate pain hypersensitivities and promote the transition from acute to chronic pain after peripheral nerve injury.

Disclosures: **J. Peng:** None. **N. Gu:** None. **L. Zhou:** None. **U.B. Eyo:** None. **M. Murugan:** None. **W. Gan:** None. **L. Wu:** None.

Poster

510. Microglia II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 510.16/T17

Topic: B.12. Glial Mechanisms

Title: The role of P2X7 in neuroinflammation: effect on mouse primary microglia and astrocytes

Authors: *N. M. TAYLOR, Y. HE, A. BHATTACHARYA;
Janssen, San Diego, CA

Abstract: P2X7, an ATP-gated cation channel, plays important roles in various inflammatory, immune, and neurologic disorders. Brain P2X7 is thought to be an important mediator of neuroinflammation: to that end, it is well established that activation of P2X7 causes release pro-inflammatory cytokines, IL-1β and IL-18. In this study we addressed the role of P2X7 in modulation of gliotransmitters outside the IL-1/IL-18 family. First, our RNA-seq data showed that P2X7 gene is expressed in glial cells (microglia and astrocytes) in both mouse and human brains. Other than IL-1α and IL-1β, A-804598 (1 μM), a P2X7 antagonist had no significant effect on the panel of gliotransmitters when murine microglia was challenged with LPS and Bz-ATP. To extend the pharmacology, we tested microglia and astrocytes harvested from either P2X7 knockout (Pfizer KO obtained under license from JAX) or wild type (WT) controls. As reported previously, the KO expressed a leaky splice variant at low levels in brain tissue. P2X7-dependent component of IL-1β and IL-18 release was absent in P2X7 KO microglia; the astrocytes did not release these cytokines. We next sought to address the role of P2X7 in microglial activation. Under basal culture condition, WT microglia and P2X7 KO microglia had similar morphology. Under inflammatory conditions induced by LPS or LPS plus IFNγ with or without Bz-ATP, microglia (both WT and KO) responded with morphological changes from a ramified-shape to an amoeboid shape indicating changes in microglial morphology was not dictated by P2X7 activation. Contrary to this, and perhaps more interesting, more P2X7 KO microglia survived with increased Iba1 expression by immunostaining when exposed to LPS plus Bz-ATP or Bz-ATP alone compared with WT microglia, suggesting that P2X7 is involved in ATP-induced microglia cell death. Taken together, our data demonstrate that P2X7 is a modulator of release of IL-1 family, and play a role in microglial cell death.

Disclosures: **N.M. Taylor:** None. **Y. He:** None. **A. Bhattacharya:** None.

Poster

510. Microglia II

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 510.17/T18

Topic: B.12. Glial Mechanisms

Support: Japanese Grant 15K20047

Title: The difference of microglial activation between in spinal posterior horn and in spinal anterior horn after peripheral nerve injury.

Authors: *T. NISHIHARA[1], J. TANAKA[2], T. YOROZUYA[1];
[1]Dept. of Anesthesia and Perioperative Med., [2]Dept. of Mollecular and cellular physiology, Ehime Univ., Toon, Japan

Abstract: After peripheral nerve injury, microglial cells become activated while directly attaching neuronal somata and eliminating synapses as known as synaptic stripping. Such activated microglia in the posterior horn in the spinal gray matter have been suspected as one of the critical causes for neuropathic pain. In the present study, we have compared the activated microglia in the posterior horn with those in the anterior horn in terms of morphology and the expression of phagocytosis-related factors after chronic constriction injury of the left sciatic nerve. First, we have comprehensively analyzed mRNA expression in the left and the right spinal cord on the 7 days after the constriction utilizing a next generation sequencer. As a result, expression of mRNA for complements C1s and C3 was the most strongly elevated among the whole mRNA species. Next, we compared mRNA expression encoding phagocytosis related factors in the left and the right anterior parts of the cord and those in the posterior parts. Compliments expression in the left cords was higher than in the rights and that in the left posterior was higher than in the left anterior. Expression of CD11b, a C3b receptor, was expressed at a higher level in the left posterior than that in the left anterior, whereas GAS6 and NG2 chondroitin sulfate proteoglycan mRNA expressions were more marked in the left anterior horn than posterior horn. Confocal laser scan microscopy revealed that microglial cells with flattened shape intimately attached to large neurons in the left anterior horn, while those in the left posterior horn displayed amoeboid-like shape and the attachment to neurons was not apparent. These results suggest that the activated microglial cells in the anterior and the posterior horns may have distinct functions in the spinal cord after peripheral nerve injuries.

Disclosures: **T. Nishihara:** None. **J. Tanaka:** None. **T. Yorozuya:** None.

Poster

510. Microglia II

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Topic: A.04. Transplantation and Regeneration

Support: P01 NS057228

RO1 NS057190

DGE 1444932

Title: Microglial impact on KCC2 in the ventral horn after peripheral nerve injury

Authors: *E. T. AKHTER[1], F. J. ALVAREZ[2], A. W. ENGLISH[3];
[1]Cell Biol. & Physiol., [2]Physiol., [3]Cell Biol., Emory Univ., Atlanta, GA

Abstract: Following peripheral nerve injury, spinal cord microglia become activated and proliferate in dorsal horn regions receiving projections from injured sensory afferents and in the ventral horn around the injured motor pool. Microglia activation contributes to the development of neuropathic pain by inducing hyperexcitability in dorsal horn neurons through downregulation of the potassium-chloride cotransporter-2, KCC2, through a BDNF dependent mechanism. Similarly, KCC2 is downregulated in motoneurons axotomized after nerve injury, but its significance and mechanisms of downregulation are unknown. We tested here whether microglia may also drive the downregulation of KCC2 on axotomized motoneurons. To investigate this relationship we injured the sciatic nerve by performing complete nerve transections in transgenic mice in which microglia express EGFP ($CX3CR1^{gfp/wt}$). We then compared the time course, level and distribution of the microglial response in the ventral horn with the degree and distribution of KCC2 depletion on injured motoneuron cell bodies and dendrites fully labeled by transfection with AAV1-mCherry. In addition, we examined the time course of KCC2 expression on the membrane of injured motoneurons (retrogradely labeled from muscle with Fast Blue or cholera toxin subunit b coupled to Alexa 555) at different times after injury, during axon regeneration and muscle reinnervation. Though the number of microglial contacts on motoneuron dendrites does not correlate with the degree of KCC2 loss, the region containing activated microglia closely overlapped with the extension of KCC2 depletion on motoneuron dendrites. The onset of KCC2 downregulation coincides with the microglial response and is restored after the response attenuates. Current experiments are performing cell-specific deletion of BDNF in microglia to test whether this manipulation blocks KCC2 downregulation in motoneurons after peripheral nerve injury.

Disclosures: E.T. Akhter: None. **F.J. Alvarez:** None. **A.W. English:** None.

Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: 2013R1A1A2074231

2013R1A2A2A01067248

SSTF-BA1502-13

Title: Polyamidoamine dendrimer-conjugated triamcinolone acetonide attenuates nerve injury-induced mechanical allodynia by inhibiting spinal cord microglia activation

Authors: *K. NOH[1], H. KIM[1], H. LIM[1], H. MIN[1], B. CHOI[1], J. OH[2], S. CHOI[2], J.-S. PARK[2], S. LEE[1];
[1]Sch. of Dent., [2]Sch. of Chem. and Mol. Engin., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Background: Accumulating evidence on the causal role of spinal cord microglia activation in the development of neuropathic pain after peripheral nerve injury (PNI) suggests that a microglia activation inhibitor might serve as an analgesic drug for neuropathic pain. Studies also show that polyamidoamine (PAMAM) dendrimer may function as drug delivery vehicle to microglia in the central nervous system. In this regard, we developed PAMAM dendrimer-conjugated triamcinolone acetonide (TA) that we have previously screened for microglia activation inhibitor, and tested its analgesic efficacy in the mouse PNI model. **Result**: PAMAM dendrimer is delivered selectively to spinal cord microglia upon i.t. administration. PAMAM dendrimer-conjugated TA (D-TA) inhibits LTA-induced proinflammatory gene expression in primary glial cells. Likewise, i.t. D-TA administration inhibited PNI-induced spinal cord microglia activation and the expression of pain-related genes in the spinal cord such as Nox2, IL-1β, TNF-α, and IL-6. D-TA administration right after nerve injury almost completely reversed PNI-induced mechanical allodynia up to 3 days. Likewise, D-TA administration 1.5 dpi significantly attenuated mechanical allodynia. **Conclusion**: Our data demonstrate that D-TA attenuates neuropathic pain after PNI by inhibiting spinal cord microglia activation, suggesting a therapeutic implication for the treatment of neuropathic pain.

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Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: NIH Grant DC011137

Title: Deafferentation and direct injury cause different microglial response profiles in the adult zebrafish olfactory bulb

Authors: *S. R. VAR, C. A. BYRD-JACOBS;
Biol. Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: The inherent plasticity of the zebrafish olfactory system serves as a useful model for examining the response of immune cells following injury. Microglia are the resident immune cells of the central nervous system that respond to damage by migrating to the site of injury and phagocytizing neuronal debris. Preliminary data suggested that direct injury to the olfactory bulb results in an acute immune response by resident microglia, while further proliferation may be due to migration of microglia from other regions of the brain or peripheral leukocytes entering through the olfactory nerves. We performed peripheral deafferentation and direct injury to the olfactory bulb in the whole fish and compared it to the isolated brain removed of all afferent input and peripheral influence. The olfactory bulbs of adult zebrafish were damaged by either cauterizing the olfactory organ or directly injuring the bulb with a stab wound. Removal of afferent input and peripheral influence was performed by isolating and culturing the brain for 4h. Mouse monoclonal 4C4 antibody was used to label microglia. Comparisons of the whole fish treatment groups to control fish revealed that there was a significant increase in activated microglia in the damaged bulb following peripheral deafferentation ($p<0.01$) and an increase in activated microglia in both the ipsilateral and contralateral bulbs following direct bulb injury ($p< 0.01$). In the whole fish, there were significant differences between active microglial profiles in peripheral deafferentation and direct injury in both the ipsilateral ($p=0.03$) and contralateral ($p<0.01$) bulbs. In the isolated brains, there were significantly more activated microglia in the olfactory bulbs after 4h in culture than in isolated brains immediately after dissection ($p=0.02$). When the isolated brain in culture received a direct injury to the right bulb, there was a significant increase in activated microglia bilaterally compared to the control ($p<0.01$); however, there were no differences between the microglial response profiles following direct bulb injury in the whole fish compared to direct bulb injury in the isolated brain. We found that peripheral deafferentation and direct injury to the olfactory bulb in the whole fish result in different microglial response profiles: a unilateral response to deafferentation and a bilateral response to direct injury. In the isolated brain, there is a significant microglial response following 4h in culture, suggesting that microglia can respond to damage without afferent input or peripheral influence. Further work is required to explore the origin and temporal sequence of the immune cells that respond to injury.

Disclosures: **S.R. Var:** None. **C.A. Byrd-Jacobs:** None.

Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: NRF 2012M3A9C6049935

DGIST Convergence Science Center Program 15-BD-04

Title: Translocator protein 18 kDa (TSPO) ligands, Ro5-4864 and PK11195, have shown anti-inflammatory effects in diverse neuroinflammatory disease models.

Authors: *J.-W. LEE, L. KIM, H.-J. SHIM, E.-K. KIM, S.-W. YU;
DGIST, Daegu, Korea, Republic of

Abstract: Neuroinflammation is related with activation of the innate immune system involving infiltrating monocytes and macrophages as well as resident microglia. Previously, we reported the anti-inflammatory effects of TSPO ligands in microglial cells in vitro and in vivo. However, the anti-inflammatory mechanisms of TSPO ligands in innate immune system still remain poorly understood. NLRP3 inflammasome activation as a part of the innate immune system, has been implicated in a variety of neuroinflammatory diseases, including Alzheimer's disease, Parkinson's disease, and depression. Here, we demonstrate for the first time that TSPO ligands, especially Ro5-4864, potently inhibits ATP-induced NLRP3 inflammasome activation in microglia and macrophages. Ro5-4864 efficiently suppressed NLRP3 translocation to mitochondria, inflammasome assembly/oligomerization, activation of caspase-1 and subsequent secretion of IL-1β and IL-18. TSPO ligands prevented mitochondrial perturbation upstream of NLRP3 inflammasome activation. Our study can provide novel insight into the anti-inflammatory mechanism of TSPO ligands through regulation of NLRP3 inflammasome activation.

Disclosures: **J. Lee:** None. **L. Kim:** None. **H. Shim:** None. **E. Kim:** None. **S. Yu:** None.

Poster

510. Microglia II

Location: Halls B-H

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Program#/Poster#: 510.22/U5

Topic: B.12. Glial Mechanisms

Title: Toll-like receptor 2 has not an important role in an immediate-early microglial reaction to two-photon laser-induced cortical injury *In vivo*

Authors: *H. YOON[1,2], Y. JANG[3], S. KIM[4], S. LEE[3], S. KIM[1];
[1]Dept. of Physiol., Kyunghee Univ. Colledge of Korean Med., Seoul, Korea, Republic of; [2]BK 21 plus, Kyunghee university Col. of Korean medicine, Seoul, Korea, Republic of; [3]Oral physiology and neuroscience, [4]Seoul national university, Seoul, Korea, Republic of

Abstract: Microglia, the resident immune cells in the central nervous system, can be rapidly activated to pathological insults. Toll-like receptor 2 (TLR2) is a pattern recognition receptor that plays a primary role in pathogen recognition and initiate of innate immunity. Although many previous studies have suggested that TLR2 contributes to microglial activation and subsequent pathogenesis following brain tissue injury, it is still unclear whether TLR2 has a role in microglia dynamics in the resting state or in immediate-early reaction to the injury *in vivo*. By using in vivo two-photon microscopy and *Cx3cr1*-^{GFP} knock in/ TLR2-knockout (KO) mice, we first monitored the motility of microglial processes (i.e. the rate of extension and retraction) in the somatosensory cortex of living TLR2-KO and WT mice; Microglial processes in TLR2-KO mice show the similar motility to that of WT mice. We further found that microglia rapidly extend their processes to the site of local tissue injury induced by a two-photon laser ablation and that such microglial response to the brain injury was similar between WT and TLR2-KO mice. These results indicate that there are no differences in the behavior of microglial processes between TLR2-KO mice and WT mice when microglia is in the resting state or encounters local injury. We suggest that TLR2 signaling does not have an essential role in the resting state microglial behavior as well as in the immediate early microglial response to a brain tissue injury *in vivo*.

Disclosures: **H. Yoon:** None. **Y. Jang:** None. **S. Kim:** None. **S. Lee:** None. **S. Kim:** None.

Poster

510. Microglia II

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Program#/Poster#: 510.23/U6

Topic: B.12. Glial Mechanisms

Title: The effects of minocycline on lps induced cytokine release.

Authors: *I. FRASER, N. TAYLOR, Y. HE, R. WYATT, A. BHATTACHARYA, P. BONAVENTURE;
Janssen, San Diego, CA

Abstract: Minocycline is known to be exhibit anti-neuroinflammatory properties. Current research is examining the possible neuroprotective and anti-neuroinflammatory effects of minocycline against the development and progression of several neurodegenerative disorders. To better understand the possible neuroprotective qualities of minocycline it is important to demonstrate this effect in both in vitro and in vivo models of neuroinflammation, in a systematic evaluation.
To this end, lipopolysaccharide (LPS) was used as an inflammatory agent to induce cytokine and chemokine release from mouse primary microglia in vitro and from rat hippocampus in vivo. We then attempted to block LPS induced cytokine/chemokine release by administering minocycline. Our studies show that minocycline (20 μM) modulates LPS (10 and 100 ng/ml) induced cytokine/chemokine release in the mouse primary microglia in vitro. However, we did not find the same effect of minocycline (80 mg/kg, i.p.) on LPS (10 mg/ml via reverse dialysis) induced cytokine/chemokine release in rat hippocampus, as measured by in vivo microdialysis. Lack of a strong effect of minocycline in vivo was not due to the absence of the compound in the brain as we show that minocycline does indeed cross the blood-brain barrier and is even sufficiently present in the brain (4 μM) during LPS exposure.

Disclosures: I. Fraser: None. **N. Taylor:** None. **Y. He:** None. **R. Wyatt:** None. **A. Bhattacharya:** None. **P. Bonaventure:** None.

Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: NIH T32 5T32HL098062-04

NIH RO1 HL-081823

Title: Microglia activation occurs early in sustained hypoxia and is necessary for ventilatory acclimatization to hypoxia

Authors: *J. A. STOKES, T. E. ARBOGAST, F. L. POWELL;
Medicine/Physiology, UC-San Diego, La Jolla, CA

Abstract: Ventilatory acclimatization to hypoxia (VAH) is a time-dependent increase in ventilation that persists upon return to normoxia and involves plasticity in central nervous system (CNS) respiratory centers and peripheral chemoreceptors. Plasticity in the CNS increases ventilatory motor output for a given chemoreceptor input but the mechanisms of such plasticity are unknown. Here we tested the role of glial cells, which are known to affect synaptic transmission and central respiratory control. We measured the time course of microglia and astrocyte activation following different durations of chronic sustained hypoxia (CSH, 10% O_2). Three to four male Sprague Dawley rats were exposed to CSH for 0.5, 1, 4 and 12 hours and 1, 4 and 7 days followed by immediate tissue perfusion and brainstem collection. We measured glial cell activation in the nucleus tractus solitarius (NTS; primary synapse in the CNS from carotid body chemoreceptors) and the hypoglossal motor nucleus (CN XII; respiratory motor neurons) using immunohistochemistry and image analysis (FIJI/ImageJ and IMARIS with Filament Tracer). After 1 hour of CSH, microglia in both the NTS and CN XII displayed shorter and fewer branches, indicating activation, but they return to a ramified morphology with more branching and with longer branches after 4 hours of CSH. Astrocytes increased glial fibrillary acidic protein (GFAP) expression in the NTS after 4 hours of CSH, but in the CN XII region GFAP expression was increased only after 7 days of CSH. Similar changes in microglia or astrocytes were not observed in adjacent non-respiratory nuclei. The number of microglia did not change with CSH in the NTS or CN XII, indicating no proliferation. Similarly, the number of astrocytes did not change in the NTS with CSH, but astrocyte number did increase in the CN XII after 7 days of CSH. Systemic administration of a microglia inhibitor (minocycline, 45mg/kg i.p.) during 7 days of CSH blocked the increase in hypoxic ventilation measured with barometric pressure plethysmography in unrestrained rats (n=3). However, once VAH was established with 4 days of CSH, minocycline treatment for 3 more days of CSH did not reverse the increase in

hypoxic ventilation (n=3). Our results support the hypothesis that microglia activation is required for the induction but not the maintenance of plasticity leading to VAH.

Disclosures: J.A. Stokes: None. **T.E. Arbogast:** None. **F.L. Powell:** None.

Poster

510. Microglia II

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 510.25/U8

Topic: B.12. Glial Mechanisms

Support: the National Natural Science Foundation of China (81171041)

the National Natural Science Foundation of China (81271217)

Title: Inhibition of astrocytes released IL15 reduces CD8 T cell mediated neuronal apoptosis in experimental traumatic brain injury in rats

Authors: *Y.-M. ZHANG[1], L. WU[1], H. WANG[1], G.-L. ZHANG[2], R. HUA[3];
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Abstract: Microenvironmental factors that activate immune cells infiltrating into CNS of rats with traumatic brain injury (TBI) remain elusive. Cytokine IL-15 is crucial in the development and activation of the $CD8^+$ T lymphocyte which is accumulated in brain region of TBI. We examined whether the IL-15 released from astrocytes is able to stimulate $CD8^+$ T lymphocyte response. We found that TBI was associated with astrocytes activation and an increase in the IL-15 level. The distribution of $CD8^+$ T lymphocytes in TBI lesion area were matched to IL-15 positive cells. Furthermore, Gra-b released by $CD8^+$ T lymphocytes induced neuronal apoptosis by activating caspase 3 and cleaving PARP. In addition, pretreatment with fluorocitrate, an inhibitor of astrocytes, decreased the expression of IL-15 positive and $CD8^+$ T cells, improved neurological function and memory, and decreased neuron apoptosis. Pretreatment with rIL-15 upregulated the levels of $CD8^+$ T cells and Gra-b, and induced neuronal death. Our data indicate that IL-15 derived from astrocytes induces neuron apoptosis by enhancing $CD8^+$ T cell function. Key words: IL-15; TBI; astrocytes; $CD8^+$ T cells; neuronal apoptosis. This work is supported by grants from the National Natural Science Foundation of China (81171041 to Y-M. Zhang, 81271217 to G. Zhang), Key Subject of Colleges and Universities Natural Science Foundation of Jiangsu Province (13KJA320001 to Y-M. Zhang).

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Poster

510. Microglia II

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 510.26/U9

Topic: B.12. Glial Mechanisms

Support: Merit Review grant (NEUD-004-07F) from the Veterans Administration

Title: Metabolic stress in microglia dysregulates sirtuin pathway and mitochondrial function

Authors: *S. PUGAZHENTHI[1], A. TYAGI[2], T. CHONG[3];
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Abstract: The immune system is recognized as an important sensor of metabolic stress, one of the modifiable risk factors of dementia. We have previously observed that metabolic stress-induced mitochondrial injury activates inflammasome formation in microglia. Sirtuins comprise a highly conserved family of NAD^+-dependent enzymes. They maintain metabolic homeostasis and reduce cellular damage and inflammation. In this study, we examined the effects of nutrient overload-induced metabolic stress on the sirtuin pathway and mitochondrial dysfunction in the Alzheimer's transgenic mouse brain and in cultured microglia.
High-fat feeding induced the expression of inflammatory pathway genes in the brain of both nontransgenic and 3XTg AD mice. Western blot analysis revealed activation of NF-kB, as shown by the decreases in the levels of IkB. Intracellular levels of Aβ in the AD mouse brain increased further following high-fat feeding. Decreases in the levels of SIRT1 and SIRT3 in the AD mouse brain were exacerbated by metabolic stress. Immunofluorescent staining showed the decrease of sirtuins to be specifically in Iba1-stained Microglia.
Palmitic acid treatment on BV2 cells, a mouse microglial cell line, caused mitochondrial fragmentation, decreased the levels of mitochondrial complexes I and II, and NF-kB activation. Examination of mitochondrial respiration measured by high resolution respirometry using Oroboros Oxygraph-2k decreased in response to the carbohydrate substrates in stages 2, 3, and 4. To delineate the role of SIRT1 in the regulation of mitochondrial function, $SIRT1^{-ve}$ BV2 cells were generated. Mitochondrial complexes I and II decreased following the silencing of SIRT1. These cells showed significantly low respiration as compared to control BV2 cells. Palmitic acid-induced NF-kB activation was also exacerbated in $SIRT1^{-ve}$ cells.
Additional studies were performed to determine the role of SIRT3 on the mitochondrial function.

Brain samples from SIRT3 knock-out mice, fed western diet, showed decreased maximum uncoupled respiration rate. SIRT3 silencing in BV2 cells also decreased mitochondrial respiration.
Our findings reveal a novel paradigm that nutrient excess-induced metabolic stress causes mitochondrial injury in the brain, leading to neuroinflammation, an important player in the pathogenesis of Alzheimer's disease. Sirtuin pathway could be a potential therapeutic target to delay cognitive decline.

Disclosures: **S. Pugazhenthi:** None. **A. Tyagi:** None. **T. Chong:** None.

Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: the Program for Promotion of Fundamental Studies in Health Sciences of NIBIO

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Title: Microglia participate in the functional maturation of blood-brain barrier by regulating cytokine-chemokine circumstances as members of neurovascular unit.

Authors: *K. SATO, Y. SHIGEMOTO-MOGAMI, K. HOSHIKAWA, Y. SEKINO;
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Abstract: There is growing evidence that the blood-brain barrier (BBB) permeability is regulated by various cells comprised of neurovascular unit (NVU). However, the relationship between microglia and BBB is largely unknown. In this study, we investigated the roles of microglia in the functional maturation process of BBB and the underlying mechanisms using in vitro BBB model comprised of endothelial cells, pericytes, and astrocytes (Pharmaco-cell co). When we added non-stimulated microglia to astrocytes in the brain side of the BBB model during the maturation period (DIV1-4), trans-endothelial electrical resistance (TEER) and the

expression level of Claudin-5, a member of tight junction proteins (TJs), were significantly increased. On the other hand, LPS-activated microglia significantly decreased the TEER and the levels of two TJ proteins (Occludin and Claudin-5). We next examined the involvement of cytokines/chemokines in the enhancement of BBB barrier function by microglia. We measured the concentrations of cytokines/chemokines in the brain side of the BBB model supplemented with non-stimulated microglia or LPS-stimulated microglia comprehensively using MAGPIX system (millipore). Among cytokines/chemokines, the concentrations of X showed a correlation with changes of BBB functions, while the concentration of Y showed an inverse correlation with the changes of BBB functions. We have confirmed that X alone significantly increased TEER but had no effects on the expression levels of TJ proteins by 4-day treatment. Currently we are examining the roles of Y. These results so far suggest that microglia regulate BBB barrier function by controlling the dynamics of cytokines/chemokines.

Disclosures: K. Sato: None. **Y. Shigemoto-Mogami:** None. **K. Hoshikawa:** None. **Y. Sekino:** None.

Poster

510. Microglia II

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Topic: B.12. Glial Mechanisms

Support: NIH R01GM076063

Title: Blockade of potassium channel Kv1.3 ameliorates brain injury in a model of infection-sensitized neonatal hypoxia-ischemia

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Abstract: Perinatal hypoxia-ischemia (HI) insult causes major damage to the developing brain and its severity is augmented by perinatal infection. In a rodent model of endotoxin (lipopolysaccharides or LPS)-sensitized hypoxic/ischemic perinatal brain injury (LPS-HI), influx of peripheral T_H17-like lymphocytes and monocytes to the brain as well as persistent microglial activation has been shown to play an essential role in such neonatal brain injury. Because the voltage-gated potassium channel Kv1.3 modulates the migration and activation of a subset of T cells (particularly T_H17 cells), monocytes, and microglia, we hypothesize that Kv1.3 blockade may ameliorate brain injury in newborns suffering from infection-sensitized HI. To test this

hypothesis, we injected P10 C57BL/6J mice with LPS (0.3 mg/kg intraperitoneally) and immediately subjected them to ischemia (left common carotid artery ligation) and hypoxia (10% O_2 at 37^oC for 40 min). We found that IBA1+ microglia/macrophages accumulated in the left (ipsilateral) cortex and hippocampus with associated neuronal loss, but not in the right hemisphere contralateral to the side of ischemia. These microglia/macrophages showed significant upregulation of Kv1.3 transcript and protein, as measured by qPCR and immunohistochemistry, respectively. The enhanced Kv1.3 channel activity/quantity was confirmed by whole-cell patch-clamp conducted on acutely isolated microglia/macrophages. We next assessed the outcomes of the LPS-HI model after Kv1.3 blockade by either administering a specific Kv1.3 blocker PAP-1 (40 mg/kg every 12 h, *i.p.*) or using Kv1.3 knockout mice in C57BL/6J background. We found that both pharmacological and genetic blockade of Kv1.3 significantly mitigated neuronal loss and microglia/macrophage accumulation in LPS-HI mice, reduced the induction of pro-inflammatory cytokines and chemokines, and improved the performance in rotarod and 14-score neurological deficit tests. Flow cytometry showed that Kv1.3 blockade reduced the populations of both CD11b+/CD45^{high} and CD11b+/CD45^{low} cells, suggesting a reduction of both activation of microglia and infiltration of monocyte-derived macrophages. Our results implicate a novel Kv1.3-orchestrated mechanism in neonatal brain injury and suggest a novel therapeutic approach.

Disclosures: **L. Jin:** None. **M. Horiuchi:** None. **H.M. Nguyen:** None. **Y. Chen:** None. **H. Wulff:** None. **I. Maezawa:** None.

Poster

510. Microglia II

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Topic: C.03. Parkinson's Disease

Support: NSFC

Title: Autophagy regulates MAVS signaling activation in a phosphorylation dependent manner in microglia

Authors: *Z. YUAN[1], J. CHENG[2], Y. LIAO[2], Y. XU[3];
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Abstract: Mitochondrial antiviral signaling protein (MAVS) signaling plays an important role in antiviral immunity and autoimmunity. However, the pathophysiological role of this signaling

pathway, especially in the brain, remains elusive. Here, we demonstrate that MAVS signaling exists in the brain and mediates poly(I:C)-induced inflammation. Importantly, *in vivo* data show that deficiency of *MAVS* prevents MPTP-induced microglial activation and dopaminergic neuron loss. Along with the MAVS signaling activation, there is an induction of autophagic activation. LC3 knockdown or *Atg5* deletion in microglial cells strengthens MAVS-mediated inflammation. Further studies show that MAVS directly binds to LC3 through a classical LIR motif $Y_{(9)}xxI_{(12)}$ Interestingly, we find that c-Abl kinase phosphorylates MAVS and increases its interaction with LC3 and tyrosine phosphorylation of MAVS is indispensible for downstream signaling activation. Together, our findings reveal the molecular mechanisms underlying the regulation of MAVS-dependent microglial activation in the nervous system, thus providing a potential target for the treatment of Parkinson's disease.

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Poster

511. Demyelinating Disorders and Their Mechanisms

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 511.01/U13

Topic: B.13. Demyelinating Disorders

Support: NIH PRJ91PG

Title: Parsing functional consequences of mutated GFAP using Alexander disease iPSCs

Authors: *J. JONES[1,2,3,4], M. DUBOVIS[1], R. BRADLEY[1], S. G. CANFIELD[2], M. HANNA[3], T. HAGEMANN[1], R. KRENCIK[4], J. AUDHYA[3], S. P. PALECEK[2], E. V. SHUSTA[2], A. MESSING[1], S.-C. ZHANG[1];
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Abstract: Alexander disease is caused by mutations in the glial fibrillary acidic protein (GFAP) gene. It remains unknown how GFAP mutations result in astrocyte malfunction and dysmyelination. We have established induced pluripotent stem cells (iPSCs) from Alexander disease (AxD) patients with different GFAP mutations. We have also generated isogenic iPSCs by correcting the GFAP mutations with CRISPR/CAS9. While both AxD and isogenic iPSCs differentiated to astrocytes to a similar degree, AxD astrocytes exhibited GFAP aggregation, a hallmark pathology of the disease. Functional analysis revealed altered calcium wave kinetics and reduced capacity to induce tight junction formation in an *in vitro* model of the blood brain

barrier. These results highlight the utility of iPSC disease modeling as a means to recapitulate human disease *in vitro.* AxD astrocytes provide a tool to reveal how the cytoskeletal protein GFAP functions in astrocytes in both health and disease.

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Poster

511. Demyelinating Disorders and Their Mechanisms

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Topic: B.13. Demyelinating Disorders

Support: The Ellison Medical Foundation AG-NS-1101-13

Shriners Hospitals for Children 85500-PHI-14

Title: PIP3/AKT activation in $NG2^+$ glial progenitors promotes oligodendrocyte differentiation and regeneration

Authors: *E. GONZALEZ FERNANDEZ[1], M. FUKAYA[2], K. HYUKMIN[1], S.-B. HAN[1], Y.-J. SON[1], S. H. KANG[1];
[1]Shriners Pediatric Hosp. Ctr., Philadelphia, PA; [2]Anat., Kitasato Univ. Sch. of Med., Sagamihara, Japan

Abstract: Oligodendrocytes (OLs) are myelin-forming glia that are critical for rapid axonal conduction and metabolic support for axons in the CNS. In the healthy CNS, homeostatic maintenance of OLs seems to be well supported by abundant $NG2^+PDGF\alpha R^+$ OL progenitors (OLPs), which can give rise to mature OLs not only during early development, but also in later life. In response to OL loss following focal demyelinating injury, OLPs generate new OLs, leading to remyelination in the adult CNS. However, in demyelinating diseases, for unknown reasons such reparative processes do not properly function. Although molecular mechanisms for early OL development have been identified, and knowledge of relevant signaling pathways has accumulated, the effective signaling pathways that can promote the OL regeneration in the mature CNS are yet to be identified.
In search of an effective targetable cell signaling that can promote OL regeneration *in vivo*, we tested the PIP3/AKT/mTOR signaling by deleting *Pten* or *Tsc1*, the two inhibitory upstream regulators of mTOR activity, selectively in OLPs, with tamoxifen-administered *PDGFαR-CreER*

mice. We also performed simultaneous fate analysis of OLPs by adding ROSA26-EYFP reporter to the *PDGFαR-CreER ± Pten (f/f)* mice. The OLP-specific *Pten* deletion resulted in enhanced proliferation $EYFP^+$ OLPs, and facilitated OL development in the two tested age windows, P20 to P41 and P45 to P75, whereas *Tsc1* deletion showed the opposite results. The robust increase in rate of OL differentiation by PTEN inactivation was more prominent in the gray matter areas than white matter areas. Moreover, after focal demyelination of the mature spinal cord (at P60), the *Pten*- deleted OLPs exhibited a significantly enhanced rate of OL regeneration. To better understand the dissimilar outcomes from deletions of *Pten* and *Tsc1*, we crossed *Pten (f/f)* mice with *Mtor (f/f)* mice. Our inducible genetic deletion of *Mtor* alone clearly decreased both the level of p70S6 kinase phosphorylation and the number of mature OLs, confirming effective gene ablation. However, the impact of *Pten* deletion was not reversed by the additional deletion of *Mtor*.
These results suggest that PTEN is an important negative regulator of the rate of OL maturation, and its inhibition (or activating PIP3/AKT) in OLPs can be an effective approach to promote OL differentiation and remyelination. Despite the known importance of mTOR signaling in OL development, the major downstream effectors of PIP3/AKT for the observed enhancement in OL differentiation appear not to include mTOR.

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Poster

511. Demyelinating Disorders and Their Mechanisms

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Topic: B.13. Demyelinating Disorders

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Title: mHTT-expressing OPCs exhibit a SOX10 and MYRF disruption-associated suppression of oligodendrocytic development and myelinogenesis and fail to differentiate as myelinating oligodendrocytes *In vivo*

Authors: *A. C. LAMPP[1], M. OSIPOVITCH[1], L. ZOU[2], A. ASENJO MARTINEZ[1], H. BURM[2], D. CHANDLER-MILITELLO[2], X. LI[2], J. WINTERMUTE[2], A. BENRAISS[2], M. S.

WINDREM[2], S. A. GOLDMAN[1,2];
[1]Ctr. For Basic and Translational Neurosci., U O Copenhagen, Kobenhavn N, Denmark; [2]Ctr. for Translational Neuromedicine, Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Huntington`s Disease (HD) is characterized by striatal and cortical neuronal degeneration, but it is also associated with myelin loss, potentially reflecting dysfunction or failed replacement of oligodendrocytes. Nonetheless, the role of oligodendroglial pathology in HD has not been well explored. We have generated human oligodendrocyte progenitor cells (hOPCs) from embryonic stem cells (hESCs), derived from either huntingtin (mHTT)-mutant hESCs or controls, used fluorescence-activated cell sorting (FACS) to isolate the cells, and performed RNA sequence analysis on their extracted mRNAs to assess mHTT-dependent changes in gene expression. We found that a coherent set of key transcription factors associated with oligodendroglial differentiation (NKX2-2, OLIG2 and SOX10), as well as with myelin biosynthesis (including MYRF, MBP, MAG, OMG, PLP1 and MOG), were significantly, and often severely, down-regulated as a function of mHTT expression (fold change >2.0: corrected $p<0.01$), in a pattern auguring defective oligodendrocytic differentiation and myelin production capacity. We confirmed this observation *in vivo* by using immunodeficient myelin-deficient *shiverer* x *rag2*$^{-/-}$ mice neonatally-engrafted with hESC OPCs, whose glial populations are largely replaced by human donor-derived glia. These human glial chimeric mice, allow the direct *in vivo* comparison of oligodendrocytic differentiation and myelin formation by mHTT vs. control hESC OPCs. We found that the mHTT OPCs manifested significantly delayed and deficient oligodendrocytic maturation, as well as substantially diminished myelination, relative to normal HTT control OPCs, as quantified stereologically by immunostaining at serial time points for OLIG2, Transferrin, and MBP. Together, these data suggest that HD OPCs is a product of an mHTT-dependent block in oligodendrocytic differentiation by affected OPCs, followed by the serial failure of those oligodendrocytes that do develop to express MYRF and those downstream MYRF-dependent mRNAs associated with myelinogenesis. These findings suggest a causal role for defective OPC differentiation in the pathogenesis of HD.

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Poster

511. Demyelinating Disorders and Their Mechanisms

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Title: Potential role of G-protein coupled receptor 3 in regulating cytokine gene expression in the T lymphocytes

Authors: *S. TANAKA, K. HIRANO, T. KAMEOKA, T. MIYAGI, Y. YANASE, I. HIDE, T. SHIRAFUJI, N. SAKAI;
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Abstract: G-protein coupled receptor (GPR) 3 belongs to a member of constitutively active Gs-coupled receptors that activate 3', -5'-cyclic adenosine monophosphate (cAMP). We have previously reported that the neuronal expression of GPR3 enhances neurite outgrowth (Tanaka et al., JBC 2007), modulates proliferation of cerebellar granule cell precursors (Tanaka et al., PLoS One 2009), and associates with neuronal survival (Tanaka et al., Neurobiol Dis 2014); however, the physiological functions of GPR3, especially in other non-neuronal cells, have not been understood. In the present study, we investigated the physiological function of GPR3 in the T lymphocytes. When Jurkat cells were treated with Phorbol 12-myristate 13-acetate (PMA) and ionomycin, the expression of GPR3 mRNA was up-regulated 3 to 6 hours after treatments. Similar results were obtained when mouse splenocytes were stimulated by PMA and ionomycin. We further examined if the induction of GPR3 in the T lymphocytes could modulate the expression of cytokines in the T lymphocytes. The expression of IL-2 mRNA was increased when the CD4+ T lymphocytes from wild-type mice were stimulated by PMA and ionomycin. However, the extent of IL-2 mRNA elevation was up-regulated in these cells from GPR3 knockout mice. To further ask the role of GPR3 in the T lymphocytes. we applied experimental autoimmune encephalomyelitis (EAE) in GPR3 knockout mice and wild-type mice. GPR3 knockout mice developed significantly milder EAE clinical score compared with wild-type mice 13 to 17 days after immunization. These results suggest a potential role of GPR3 in modulating cytokine expression in the T lymphocytes, thereby affecting pathophysiology of autoimmune diseases.

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Poster

511. Demyelinating Disorders and Their Mechanisms

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Shriners Research foundation 86300

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Title: Novel role of protein tyrosine phosphatase sigma in pathogenesis of autoimmune CNS demyelination

Authors: *Y. OHTAKE[1], W. KONG[2], D. GANEA[2], S. LI[1];
[2]Microbiology&Immunology, [1]Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Protein tyrosine phosphatase sigma (PTPσ) is expressed in both the nervous and immune systems and appears to play critical roles in neurodevelopment, axon regeneration and inflammatory responses. Here, we studied the function of PTPσ in mediating inflammatory reactions in the CNS and pathogenesis of demyelination using experimental autoimmune encephalomyelitis (EAE) mouse, a model of multiple sclerosis. PTPσ deletion remarkably increased the clinical EAE symptoms and induced severe demyelination and axonal loss compared with littermate wild-type BALB/c mice immunized with myelin oligodendrocyte glycoprotein (MOG) peptide. Gene expression analysis displayed enhanced levels of several pro-inflammatory cytokines and chemokines in the spinal cord of PTPσ$^{-/-}$ mice after MOG immunization. Ex vivo experiments indicated that splenocytes derived from PTPσ$^{-/-}$ EAE mice produced higher levels of IFNγ and IL17 than those from EAE controls. Importantly, the conventional dendritic cells (cDCs), but not CD4+ T cells, expressed high levels of PTPσ and the cDCs from PTP$^{-/-}$ mice exhibited the inflammatory phenotype characterized by enhanced expression of pro-inflammatory cytokines, chemokine receptors and migration capacity, and decreased expression of anti-inflammatory cytokines. Accordingly, adoptive transfer of PTPσ$^{-/-}$ DCs induced more severe EAE disease than that of wild-type DCs. Moreover, by using a lysolecithin-induced focal demyelination model, we found that PTPσ deficiency prevented remyelination of the spinal cord axons by accumulating microglia and reactive astrocytes and reducing generation of NG2-positive oligodendrocyte progenitors. We conclude that PTPσ plays a critical role in development of autoimmune CNS inflammation by regulating functions of DCs

and other cell types and that it may become a molecular target for treating autoimmune diseases, including multiple sclerosis.

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Poster

511. Demyelinating Disorders and Their Mechanisms

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Title: Selective deletion of AMPA receptors on oligodendrocytes prevents excitotoxicity in EAE

Authors: *K. S. EVONUK[1], R. E. DOYLE[2], C. E. MOSELEY[3], H. MONYER[4], T. M. DESILVA[1];
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Abstract: Multiple sclerosis (MS) is a neuroinflammatory disease defined by demyelination and axonal damage. The most common form of MS is relapsing-remitting (RRMS), whereby exacerbations of symptoms are followed by a period of remission. A relapse is defined as a clinical event involving the onset of neurological symptoms caused by CNS inflammation. However, the number of inflammatory events in the CNS of MS patients far outnumbers clinical relapses. Current treatments for the disease are immunomodulatory and fail to fully eliminate these inflammatory events and relapses. Therefore, discovery of treatments that directly protect the CNS during immune cell infiltration is of high importance. Patients with MS have elevated

levels of glutamate in the brain as measured by MRI, implicating excitoxic mechanisms in CNS damage. Our laboratory recently showed that inhibition of a source of excitotoxic glutamate, the system x_c^- transporter, after disease onset in an animal model of MS results in reduced clinical symptoms and demyelination. To further explore the target of excitotoxic glutamate, the AMPA-type glutamate receptors (AMPARs) were selectively deleted on oligodendrocytes since numerous cell types in the CNS and immune system also express AMPARs. Using conditional knockout mice with two different oligodendrocyte promoters, we found that selective deletion of AMPARs on oligodendrocytes in adult C57Bl/6 mice subjected to experimental autoimmune encephalomyelitis (EAE) results in improved clinical scores and reduced myelin damage in the thoracic and lumbar regions of the spinal cord. To confirm that the abrogation of disease activity was CNS-specific, analysis of CNS-infiltrating immune cells by flow cytometry was performed and no changes were found in knockout mice compared to littermate controls. To further exclude changes in peripheral immune cell activity, T cell proliferation in spleens was assessed and no changes were found. These data support that AMPA receptor expression on oligodendrocytes mediates excitotoxic damage in autoimmune demyelination providing potential therapeutic implications for CNS protection in MS.

Disclosures: K.S. Evonuk: None. **R.E. Doyle:** None. **C.E. Moseley:** None. **H. Monyer:** None. **T.M. DeSilva:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 511.07/V1

Topic: B.13. Demyelinating Disorders

Title: Efffects of microtubule destabilizing drugs on CNS Myelination

Authors: *I. V. DEYNEKO[1,2], H. M. MORAN[1], M. M. URBANSKI[1], C. V. MELENDEZ-VASQUEZ[1];
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Abstract: The glial cells of the central (CNS) and peripheral (PNS) nervous systems, known as oligodendrocytes (OL) and Schwann cells (SC) respectively, wrap axons in a membrane called myelin. Nerve impulses are propagated in myelinated axons via saltatory conduction, which increases the speed of transmission and promotes efficient information processing. Loss of myelin in diseases such as multiple sclerosis in the CNS and Guillain-Barrè Syndrome in the PNS results in significant cognitive and physical impairments. Remyelination by OL and SC has been found to reverse demyelination damage and protect axons from further degeneration. Our

lab focuses on the study of the cytoskeletal proteins as a tool for promoting myelin formation and repair. Specifically, we are investigating the role of Microtubules (MTs) in the process of myelination. MTs are heterodimer polymers made up of α- and β-tubulin, which are involved in the transport of myelin proteins as well as in the formation of processes, and are thus important for the proper assembly of myelin sheaths. We are currently examining a MT-modifying drug that inhibits detyrosination of α-tubulin, a modification that affects the rate of transport along MTs as well as makes the MT more dynamic. We have found that the drug promotes the formation of myelin sheathes by SCs and OLs in co-cultures, with higher rates of branching in OPC-only cultures. Currently we are investigating the mechanisms responsible for these effects.

Disclosures: I.V. Deyneko: None. **H.M. Moran:** None. **M.M. Urbanski:** None. **C.V. Melendez-Vasquez:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 511.08/V2

Topic: B.13. Demyelinating Disorders

Support: Canadian Institute of Health

Canadian MS Society

Title: Myelinogenic plasticity of oligodendrocyte precursor cells following CNS trauma

Authors: *P. L. ASSINCK[1], G. J. DUNCAN[1], J. R. PLEMEL[2], M. J. LEE[1], D. CHUNG[1], D. SYKORA[1], J. LIU[1], D. BERGLES[3], W. TETZLAFF[1];
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Abstract: Contusive spinal cord injury (SCI) results in considerable demyelination of spared axons, which impairs signal transduction and may leave axons vulnerable to degeneration. Both oligodendrocytes (OL)s and Schwann cells remyelinate denuded axons in the subsequent weeks and months following SCI. NG2 cells, characterized by the near ubiquitous co-expression of platelet derived growth factor receptor α (PDGFRα) in the uninjured central nervous system (CNS), are oligodendrocyte progenitors (OP)s which may serve as a source of new OLs following SCI. PDGFRα-CreERT mice were crossed with Rosa26-YFP mice and administered tamoxifen to label OPs two weeks prior to contusive thoracic spinal cord injury. In the uninjured spinal cord we found that YFP was expressed in NG2+ OPs at very high efficiency, as well as

αSMA+ pericytes and fibronectin+ fibrocytic cells in the spinal roots. Following injury, many recombined cells continue to express the PDGFRa+, Olig2 and NG2, indicative they have remained as OPs, but substantial differentiation into new mature oligodendrocytes (CC1+) was observed, responsible for de novo ensheathment of >30% of the myelinated axons by three months. Strikingly, the majority of P0+ Schwann cells in the spinal cord expressed YFP, suggesting they originated from central nervous system PDGFRα+ OPs. This result was confirmed using Olig2-CreERT: Rosa26-YFP mice. Furthermore, we found that αSMA+ pericytes did not give rise to myelinating cells after SCI and that only a small proportion of P0+ myelinating cells from the spinal roots migrated into the cord and contributed to Schwann cell myelination after SCI. Overall, this work reveals enormous phenotypic plasticity of PDGFRα precursors following spinal cord injury as a source of the new remyelinating Schwann cells and oligodendrocytes in the injured spinal cord.

Disclosures: **P.L. Assinck:** None. **G.J. Duncan:** None. **J.R. Plemel:** None. **M.J. Lee:** None. **D. Chung:** None. **D. Sykora:** None. **J. Liu:** None. **D. Bergles:** None. **W. Tetzlaff:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

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Program#/Poster#: 511.09/V3

Topic: B.13. Demyelinating Disorders

Support: DMC Foundation Grant RG2015-1238

NMSS Grant RG 4906A9

NIH, NINDS Grant NS43783

Title: Electroencephalographic analysis in a metabolic stress model of Multiple Sclerosis

Authors: *C. R. RICHARDSON[1], D. Z. RADECKI[1], A. GOW[1,2,3];
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Abstract: Multiple sclerosis (MS) is a neurodegenerative disease characterized by white and grey matter lesions that contribute to a variety of disabling symptoms. MS has previously been characterized as a primary immune-mediated disease; however, emerging research suggests the involvement of metabolic stress in MS disease progression. Data from MS patient postmortem samples shows multiple phenotypes associated with clinical symptoms, including metabolic stress in white and gray matter. Our *OBiden* (*OBi*) mouse model of MS pathology involves

metabolic stress as the etiology. Through behavioral, humoral, and electrophysiological analysis we compare the pathology of *OBi* mice to that of MS patient autopsy samples to determine if our novel mouse model has pathophysiologic relevance to MS. Common symptoms for MS patients include memory loss, depression, anxiety, sleep deprivation, and lack of thermoregulation. In addition to white matter lesions and innate immune cell activation, we find hippocampal and cortical pathology, memory deficits and depression endophenotypes in *OBi* mice. We have examined the major functions of three hypothalamic nuclei and are able to rule out significant contribution of this brain region to *OBi* phenotypes. We now focus on hippocampal and cortical pathology using electroencephalography (EEG) and demonstrate reduced interhemispheric theta band coherence in 12 but not 6-month old *OBi* mice. Our behavioral testing reveals memory deficits at 12 months and depression-like endophenotype at 6 and 12 months, and we find extensive hippocampal and cortical damage at 12 months in these mutants. Correlations between interhemispheric coherence and behavioral phenotype onset suggest a common source for behavioral and coherence changes, that of hippocampal pathology. Using EEGs to further analyze hippocampus and cortex we aim to better understand the spatial and temporal emergence of pathology in these regions. We hypothesize that disturbances in interhemispheric network theta band activity are associated with the endophenotypes we observe in *OBi* mice. To investigate brain region specific contributions to the pathology we are using microelectrode arrays to interrogate cortical column level activity of layer 5 output neurons for grey matter lesion mapping and site specific identification of coherence changes. Our current data spanning behavioral, humoral, and electrophysiological approaches suggest a disease model arising through metabolic stress that is comparable to MS induction. Thus, this novel model will likely provide insight into MS pathogenesis and new opportunities for the development of disease-modifying therapies for MS patients.

Disclosures: **C.R. Richardson:** None. **D.Z. Radecki:** None. **A. Gow:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

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Topic: B.13. Demyelinating Disorders

Support: NIH Grant NS086812

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Title: Schwann cell-specific deletion of the endosomal phosphatidylinositol 3-kinase Vps34 leads to impaired radial sorting of axons and failed myelination

Authors: *F. L. ROBINSON[1], A. M. LOGAN[2], D. C. ROBINSON[2], A. F. CONDON[2], E. J. SCHMIDT[2];
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Abstract: The PI 3-kinase Vps34 (Pik3c3) synthesizes phosphatidylinositol 3-phosphate (PI3P) on endosomes, where the lipid regulates membrane traffic and macroautophagy. The dysregulation of PI3P (and its derivatives, $PI(3,5)P_2$ and PI5P) is implicated as a cause of hereditary demyelinating neuropathy (Charcot-Marie-Tooth disease; CMT). We have investigated the Schwann cell autonomous role of Vps34 in axonal sorting and myelination using mice. Schwann cells lacking Vps34 are defective in radial axonal sorting, an impairment which leads to prolonged retention of large axons in bundles and delayed myelination. In addition, Vps34-deficient Schwann cells are impaired in the elaboration of myelin, despite having reached the promyelinating stage. Loss of Vps34 in Schwann cells leads to abnormalities in both endosomal-lysosomal trafficking and autophagic flux. We also investigated how the opposed enzymatic activities of PI 3-kinases and 3-phosphatases regulate the levels of PI3P in Schwann cells. Myotubularin-related (Mtmr) 2 and Mtmr13 form a PI 3-phosphatase complex implicated in the regulation of PI3P and $PI(3,5)P_2$ in Schwann cells. Loss of either *MTMR2* or *MTMR13* causes CMT4B, a severe neuropathy characterized by distinctive myelin outfoldings. We find that the elimination of Vps34 from Schwann cells, a manipulation predicted to reduce PI3P and $PI(3,5)P_2$ levels, does not reduce dysmyelination in *Mtmr13*-deficient mice, suggesting that lowering PI3P levels may not be a viable strategy for therapeutic intervention in CMT4B. Our findings indicate that the PI 3-kinase Vps34 is critical for radial axonal sorting and myelination, and also provide insight into the complexities of the regulation of PI3P and its derivatives in Schwann cells.

Disclosures: F.L. Robinson: None. **A.M. Logan:** None. **D.C. Robinson:** None. **A.F. Condon:** None. **E.J. Schmidt:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 511.11/V5

Topic: B.13. Demyelinating Disorders

Support: Facial and Pain

Title: Altered cholesterol trafficking in mice deficient in peripheral myelin protein 22

Authors: *Y. ZHOU[1], S. LEE[2], A. I. FETHIERE[2], H. TAVORI[3], S. FAZIO[3], A. CORONA[4], G. E. LANDRETH[4], L. NOTTERPEK[2];
[1]Neurosci., [2]Univ. of Florida, Gainesville, FL; [3]Oregon Hlth. and Sci. Univ., Portland, OR; [4]Case Western Reserve Univ., Cleveland, OH

Abstract: In the peripheral nervous system, myelin, a lipid-enriched multilayered insulator of axons, is formed by Schwann cells. The correct expression of specific proteins and lipids is critical for normal myelination and stability. Rodents and humans with haplodeficiency in peripheral myelin protein 22 (PMP22), a Schwann cell glycoprotein, are prone to compression-induced myelin damage, whereas the complete absence of PMP22 in mice is associated with early death. While the precise role of PMP22 in the plasma membrane is not known our recent results indicate involvement in the establishment of lipid rafts (Lee et al., 2014). As cholesterol is a critical component of lipid rafts, here we investigated cholesterol trafficking in the absence of PMP22 using cells and tissue from PMP22-deficient mice. Morphological examination of liver from PMP22-deficient mice shows hepatosteatosis as detected by Oil red O staining, and abnormal clustering of filipin-cholesterol. In cultured primary hepatocytes from wild type animals, filipin-cholesterol is localized at zonula occludens (ZO1) reactive cell junctions, a pattern that is absent in samples from PMP22-deficient rodents. The Low-density lipoprotein receptor (LDLR), the main receptor that functions in extracellular cholesterol endocytosis, is maintained at normal levels in the liver and nerves from PMP22-deficient mice. Furthermore, the tracing of LDL-endocytosis *in vitro* using a BODIPY-LDL complex suggests cholesterol uptake by Schwann cells is not affected in the absence of PMP22. Concerning the cholesterol efflux pathway, the expression of the ATP-binding cassette transporter (ABCA1) is significantly elevated in the liver, in cultured hepatocytes, and in nerves and Schwann cells of affected mice. Conversely, the expression and processing of PMP22 are altered in nerve samples from ABCA1-deficient rodents. At steady-state, in the absence of ABCA1, PMP22 expression is elevated, and only around 50% of PMP22 contains complex carbohydrate modification, in comparison to over 80% in nerve samples from wild-type mice. Taken together these findings suggest a novel role for PMP22 in cholesterol trafficking through the interplay with ABCA1.

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Poster

511. Demyelinating Disorders and Their Mechanisms

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Program#/Poster#: 511.12/V6

Topic: B.13. Demyelinating Disorders

Support: CONACyT Grant 2015-01465

CONACyT Grant 268062

Title: Diphenylhydantoin increases proliferation of neural precursor cells and oligodendrocyte differentiation and enhances p-EGFR and p-FGFR expression in the sub ventricular zone.

Authors: *O. GONZALEZ-PEREZ[1], A. GALVEZ-CONTRERAS[2], R. GONZALEZ CASTANEDA[2], T. CAMPOS-ORDONEZ[1], V. LOPEZ-VIRGEN[1], N. MOY-LOPEZ[1], J. GUZMAN-MUNIZ[1], Z. AGUIRRE-TABOADA[1];
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Abstract: Diphenylhydantoin, also known as diphenylhydantoin, is an antiepileptic drug that induces cell proliferation in several tissues such as: heart, bone, skin, oral mucosa and neural precursors. Some of these effects appear to be mediated via fibroblast growth factor (**FGFR**) and epidermal growth factor receptor (**EGFR**). These receptors are strongly expressed in the adult ventricular-subventricular zone (**V-SVZ**), the main neurogenic niche in the adult brain. The aim of this study was to determine the proliferating cell lineage and cell fate of V-SVZ neural progenitors expanded by diphenylhydantoin, as well as the effects of this drug on EGFR/FGFR phosphorylation. Male Balb/C mice received 10 mg/kg diphenylhydantoin by oral cannula for 30 days and 100 mg/kg BrdU i.p. 24 h before sacrifice. We analyzed the proliferation of V-SVZ neural progenitors by immunohistochemistry and western blot. diphenylhydantoin-treated animals showed ~two-fold increase in the phosphorylation in the V-SVZ as compared to controls. We also found that diphenylhydantoin treatment increases the number of BrdU+/Sox-2+ and BrdU+/DCX+ cells in the V-SVZ and expands the population of Olig2-expressing cells around the lateral ventricle. After diphenylhydantoin removal, we found a high number of BrdU+/RIP+ in the olfactory bulb of the diphenylhydantoin group. In conclusion, diphenylhydantoin enhances the phosphorylation of FGFR and EGFR and promotes the expression of neural precursor markers in the V-SVZ. Upon diphenylhydantoin removal, the number of BrdU+ oligodendrocytes increases, whereas the proportion of BrdU+/NeuN+ neurons decreases in the olfactory bulb. In conclusion, diphenylhydantoin enhances the phosphorylation of FGFR and EGFR and promotes the expression of neural precursor markers in the V-SVZ. In parallel, the number of oligodendrocytes increases significantly after diphenylhydantoin removal. **Keywords:** Neural stem cells; Epidermal growth factor receptor; Fibroblast growth factor receptor; Sox2; oligodendrocyte; Olig2; Diphenylhydantoin.

Disclosures: O. Gonzalez-Perez: None. **A. Galvez-Contreras:** None. **R. Gonzalez Castaneda:** None. **T. Campos-Ordonez:** None. **V. Lopez-Virgen:** None. **N. Moy-Lopez:** None. **J. Guzman-Muniz:** None. **Z. Aguirre-Taboada:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

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Topic: B.13. Demyelinating Disorders

Support: UK MS Society Grant

The Sackler Studentship

James Baird Fund

CIMR Flow Cytometry Core Facility

Title: Exploring fate choice in demyelinating oligodendrocyte progenitor cells through single cell gene expression analysis

Authors: *N. A. MURPHY[1,2], C. MCCLAIN[1,2,3], A. WILKINSON[4], S. NESTOROWA[4,2], V. MOIGNARD[4,2], F. J. CALERO-NIETO[2,4], B. GOTTGENS[4,2], R. J. M. FRANKLIN[2,1]; [1]Clin. Neurosciences, Univ. of Cambridge, Cambridge, United Kingdom; [2]Stem Cell Inst., Wellcome Trust Med. Res. Council, Cambridge, United Kingdom; [3]Dept. of Physics, Cavendish Lab., Cambridge, United Kingdom; [4]Dept. of Hematology, Cambridge Inst. for Med. Res., Cambridge, United Kingdom

Abstract: Remyelination, the restoration of myelin sheaths around axons denuded by a demyelinating insult, is a unique regenerative process occurring in the Central Nervous System (CNS) of vertebrates. At the core of this process is the adult oligodendrocyte progenitor cell (aOPC), a multipotent cell widespread throughout the CNS. Although the presumed default fate of the aOPC is to become a myelinating oligodendrocyte, fate-mapping studies have shown that in the appropriate environment, the OPC is also capable of becoming an astrocyte or a Schwann cell. How and why these fate choices arise is currently unknown. We hypothesized that each OPC responding to a demyelinating insult will have a pattern of gene expression reflecting and in part determining its ultimate fate. Therefore, interrogating the transcriptional profile of single OPCs would not only reveal their fate, but also provide clues about the mechanism of fate determination.

Using a rodent focal toxic demyelinating model (the rat ethidium bromide caudal cerebellar peduncle lesion), we have designed a protocol to acutely interrogate gene expression in single remyelinating aOPCs using single cell RNAseq and single cell high throughput qRT-PCR. We have compared these expression profiles to those of aOPCs residing within unlesioned white matter of the the same animal, and the white matter of unlesioned animals.

Our studies have revealed marked heterogeneity within remyelinating aOPCs, and revealed both

similarities and differences in gene expression between aOPCs in the immediate and distant demyelinated environment, and those in intact white matter. We have identified a subpopulation of aOPCs expressing markers typical for astrocytic and Schwann cell fate. We are currently analyzing their transcriptome in search for mechanistic determinants and pathways associated with this fate choice, and are hoping to confirm these findings in the immediate future.

Disclosures: **N.A. Murphy:** None. **C. McClain:** None. **A. Wilkinson:** None. **S. Nestorowa:** None. **V. Moignard:** None. **F.J. Calero-Nieto:** None. **B. Gottgens:** None. **R.J.M. Franklin:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

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Title: The function of microRNA-33 in the brain and its implication for brain diseases

Authors: ***H. YOON**[1,2], J. KIM[2], K. C. BELMONTE[2], J. KIM[2];
[1]Neurobio. of Dis., Mayo Grad. Sch., Jacksonville, FL; [2]Dept. of Neurosci., Mayo Clin., Jacksonville, FL

Abstract: MicroRNAs are small noncoding RNAs that regulate gene expression either by inducing mRNA degradation or by inhibiting translation. ATP-binding cassette transporter A1 (ABCA1) is a cholesterol transporter that has been studied as a therapeutic target for Alzheimer's disease (AD). Recently, we demonstrated that microRNA-33 (miR-33) regulates the expression of ABCA1 in the peripheral tissues and in the brain. More importantly, we also found that

inhibition of miR-33 increased the ABCA1 level and cholesterol efflux, leading to decrease in the cortical Aβ level. Our *in vivo* and *in vitro* data strongly suggested that inhibition of miR-33 may be a promising therapeutic strategy for AD. To further investigate the novel functions of miR-33 in the brain, we performed an unbiased large scale gene expression study. Microarray data analysis of the mir-33 gene deficient brain samples revealed that 42 genes were up-regulated and 168 genes were down-regulated (fold change>1.35, p-value<0.05). The systemic analyses of the differentially expressed genes suggested that mir-33 may be involved in the regulation of myelination. Furthermore, we were able to predict putative direct targets of miR-33 based on the publicly available prediction algorithms and the up-regulated genes from our array data. Our studies may provide a new insight into the role of miR-33 in the brain.

Disclosures: H. Yoon: None. **J. Kim:** None. **K.C. Belmonte:** None. **J. Kim:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

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Program#/Poster#: 511.15/V9

Topic: B.13. Demyelinating Disorders

Support: RO1AG039452

R37NS34467

R37AG23084

Title: Pericyte deficiency leads to white matter demyelination and axonal degeneration

Authors: *A. M. NIKOLAKOPOULOU, A. MONTAGNE, Z. ZHAO, G. SI, D. LAZIC, A. P. SAGARE, B. V. ZLOKOVIC;
Physiol. and Biophysics, USC, Los Angeles, CA

Abstract: White matter damage is a characteristic of a plethora of neurodegenerative diseases, such as Alzheimer's disease (AD) and Multiple Sclerosis (MS). Pericytes play an important role in sustaining blood brain barrier (BBB) permeability and cerebral blood flow (CBF), while they participate in the clearance of toxic byproducts. Pericyte loss has been implicated in BBB breakdown, neuronal degeneration, cognitive impairment, inflammation, synaptic loss and has negative effects on CBF. In this study, we used $Pdgfr\beta^{F7/F7}$ mice to examine the effects of pericyte deficiency on white matter integrity, and more specifically, in the corpus callosum. Our data show that pericyte deficiency leads to early BBB leakage, which precedes demyelination and axonal degeneration; at early stages in adulthood (4wks old) animals present accumulation

of blood byproducts (IgG and fibrin), however they show no defects in myelin and axonal integrity yet. On the other hand, adult *Pdgfrβ*$^{F7/F7}$ mice (12-16wks old) exhibit a decrease in myelin thickness, as shown by electron microscopy, and a reduction in axonal and myelin-related protein expression, both of which deteriorate with aging. Furthermore, these animals exhibit behavioral deficits related to white matter damage. Previous studies have shown that hypoxia can induce coagulation and thus the formation of fibrin deposits, events that are responsible for oligodendrocyte death. Our in vitro oligodendrocyte studies show that oxygen/glucose deprivation (OGD) and fibrin accumulation can cause mature oligodendrocyte death, hence implying that the events following BBB leakage are responsible for myelin destruction. To strengthen our results, we show that fibrinogen depletion can restore myelin integrity and reverse axonal damage. Taken together, our results show that pericyte deficiency causes BBB leakage at very early stages, followed by myelin destruction and axonal degeneration, thus emphasizing the importance of the neurovascular unit in health and disease.

Disclosures: **A.M. Nikolakopoulou:** None. **A. Montagne:** None. **Z. Zhao:** None. **G. Si:** None. **D. Lazic:** None. **A.P. Sagare:** None. **B.V. Zlokovic:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

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Topic: B.13. Demyelinating Disorders

Support: MIUR, PON 'Ricerca e Competitività 2007 - 2013' project PON01_02512

Regione Veneto project protocol 103173COF/14/LR52001C2/000051

Title: A co-ultramicronized N-palmitoylethanolamine/luteolin composite promotes oligodendrocyte precursor cell survival and development and improves outcome in experimental autoimmune encephalomyelitis

Authors: ***S. D. SKAPER**[1], M. BARBIERATO[2], L. FACCI[2], G. CONTARINI[2], C. MARINELLI[2], M. ZUSSO[2], P. GIUSTI[2];
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Abstract: Oligodendrocytes, the myelin-producing cells of the CNS have limited ability to repair damage either to themselves or to other nerve cells. Such is the case in multiple sclerosis (MS), a chronic CNS neuroinflammatory demyelinating disorder. MS lesions are characterized by the presence of a compromised pool of undifferentiated oligodendrocyte precursor cells

(OPCs) which fail to mature into myelin-producing oligodendrocytes. An attractive strategy may thus be to replace lost oligodendrocytes and/or promote their maturation or proliferation. N-palmitoylethanolamine (PEA), an endogenous fatty acid amide signaling molecule possesses analgesic, anti-inflammatory, and neuroprotective actions. Recent studies show a co-ultramicronized composite of PEA and the flavonoid luteolin (co-ultraPEALut, 10:1 by mass) to be more efficacious that PEA alone in improving outcome in CNS injury models. Here, we examined the effects of co-ultraPEALut on the survival and development of OPCs isolated from newborn rat cortical mixed glial cell cultures. OPCs were maintained under conditions which favored either proliferation (FGF-2 and PDGF-AA-supplemented serum-free medium ('SFM')) or differentiation (Sato's medium containing T3 and T4). OPCs cultured in SFM displayed high expression of PDGF receptor alpha gene and improved survival in the presence of 10 μM co-ultraPEALut and down-regulation of *Apoe*, whose deletion reportedly leads to a later time of peak symptoms/disease severity and less severe demyelination/axonal damage in myelin oligodendrocyte glycoprotein (MOG35-55)-induced experimental autoimmune encephalomyelitis (EAE) in female C57BL/6 mice. In Sato's medium OPCs showed a rapid decrease in PDGF receptor alpha expression and time-dependent rise in myelin basic protein (MBP) expression. In these conditions co-ultraPEALut (10 μM) enhanced OPC morphological complexity, protein content, and gene expression for MBP, proteolipid protein and 2',3'-cyclic nucleotide 3'-phosphodiesterase, as well as genes coding for enzymes involved in cholesterol and fatty acid synthesis. Co-ultraPEALut also increased OPC content of MBP, while protecting against TNF-α-induced suppression of MBP gene expression. Moreover, co-ultraPEALut improved the clinical score in this EAE mouse model, which is often used as a chronic first-pass model of MS. Hence, strategies intended to promote endogenous remyelination in MS should focus on both enhancing the long-term survival of OPCs and on stimulating these cells to proliferate and differentiate into remyelinating oligodendrocytes. Within this context, co-ultraPEALut may represent a novel pharmacological approach.

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Poster

511. Demyelinating Disorders and Their Mechanisms

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Program#/Poster#: 511.17/V11

Topic: B.13. Demyelinating Disorders

Support: Multiple Sclerosis Research Australia

Title: The TAM receptor Tyro3 is a regulator of central nervous system myelination

Authors: ***M. D. BINDER**[1], R. AKKERMANN[2], A. A. PERERA[1], H. BUJALKA[2], A. APRICO[1], J. FIELD[1], T. J. KILPATRICK[1];
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Abstract: There is an emerging consensus that the progression of disability in MS correlates with the accumulation of axonal degeneration, which in turn is influenced by the extent of demyelination and the loss of oligodendrocytes. Therefore there is a clear need to identify and validate targets that can form the basis for the development of novel treatments that can potentiate remyelination and either slow or prevent disability progression in Multiple Sclerosis. We have previously identified that Gas6, a ligand for a family of tyrosine kinase receptors known as the TAM (Tyro3, Axl and Mertk) receptors, directly increases myelination *in vitro*, and that loss of Gas6 leads to increased disease severity and to delayed recovery in an animal model of CNS demyelination. We therefore hypothesised that the pro-myelinating effect of Gas6 is mediated through Tyro3. In order to test this hypothesis we utilised mice which were deficient in this receptor ($Tyro3^{-/-}$). We found that developmental myelination was delayed in these mice compared with WT littermates, and that oligodendrocytes derived from these mice have a reduced capacity to myelinate neurons *in vitro*. Further, we identified extracellular signal related (Erk) 1 as downstream target of the Gas6-Tyro3 axis in oligodendrocytes. In summary, we have shown, for the first time, that Tyro3 is an important regulator of myelination by oligodendrocytes, and this effect may be mediated by activation of Erk1.

Disclosures: **M.D. Binder:** None. **R. Akkermann:** None. **A.A. Perera:** None. **H. Bujalka:** None. **A. Aprico:** None. **J. Field:** None. **T.J. Kilpatrick:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

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NIH T32ES007148

NMSS RG 4257B4/1

Title: The selective mGluR group I agonist, 2-chloro-5-hydroxyphenylglycine, increases BDNF and oligodendrocyte differentiation following cuprizone-induced demyelination

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Abstract: The demyelinating agent cuprizone (0.2%) causes a decrease in brain-derived neurotrophic factor (BDNF) and myelin proteins in mice after 4 weeks of treatment. Similarly, patients with relapsing-remitting multiple sclerosis have decreased levels of BDNF in addition to decreased myelin proteins. Therefore, an intriguing therapeutic approach for these types of demyelinating diseases may be to enhance the endogenous source of BDNF. In previous studies, we found that the Group I and Group II mGluR agonist, ACPD, was able to enhance the expression of BDNF and myelin proteins when injected directly into the corpus callosum following demyelination. The aim of this study was to expand this concept to the selective mGluR Group I agonist, 2-chloro-5-hydroxyphenylglycine (CHPG), by using the more clinically relevant approach of a peripheral injection. Cuprizone or identically processed control feed was fed to mice for 4 weeks starting at 8 weeks of age prior to intraperitoneal injections of saline vehicle or CHPG (20 or 40 mg/kg). Western blot was used to analyze the levels of BDNF, myelin proteins, and oligodendrocyte progenitor cell markers. CHPG increased levels of BDNF and myelin proteins 24 hours after injection and this effect lasted up to 3 days. Myelin proteins increased without increases in CC1+ oligodendrocytes, suggesting that CHPG increases myelin proteins per cell. Additionally, cuprizone treatment causes an increase in oligodendrocyte progenitor cell markers NG2 and PDGFRα, which were reduced after CHPG administration. Interestingly, CHPG did not alter these proteins or myelin proteins in control-fed mice. Taken together, these data suggest that selective mGluR Group I agonists such as CHPG may be a therapeutic approach for treating demyelinating diseases by increasing the levels of BDNF and myelin proteins possibly by promoting differentiation of oligodendrocyte progenitor cells.

Disclosures: **K.S. Saitta:** None. **Y. Huang:** None. **C. Varghese:** None. **C. Dreyfus:** None.

Poster

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FISM Fellowship 2014/B/5 to LP

Fondazione Umberto Veronesi Fellowship to CP

Title: A promiscuous interaction between GPR17 and SDF1 may be at the basis of impaired remyelination in demyelinating diseases

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Abstract: The G-protein-coupled receptor (GPCR) GPR17 is a key player in oligodendrogenesis and a promising target for demyelinating diseases [Fumagalli et al, 2015 Neuropharmacology]. A spurious pharmacology has been reported for both GPR17 and some phylogenetically related GPCRs, including chemokine receptors (CXCRn). Specifically, GPR17 can be activated by several different molecules, including extracellular nucleotides, cysteinyl-leukotrienes and oxysterols acting as emergency signals in local reparative processes, inflammatory reactions and neurodegenerative disorders [Ciana et al 2006, Embo J; Sensi et al 2014, Cell Signal]. Whether GPR17 can be also activated by typical CXCRn ligands like Stromal Derived Factor 1 (SDF1) has not been studied before. Of note, GPR17 is necessary to start oligodendrocyte precursor cells (OPC) differentiation, but, at subsequent maturation stages, it has to be downregulated to allow cells to start myelination.
Here, we analyzed the potential interaction between SDF1 and GPR17 and its involvement in demyelinating diseases. In vitro studies, in both a classical reference assay for GPCR activity and in a model of primary OPCs maturation, showed that SDF-1 can indeed promote OPC transition to fully mature cells by directly acting as a GPR17 agonist. This interaction was further unveiled by computational approaches showing two distinct binding sites for SDF1 on GPR17, like for peptide receptors.
These data provide the basis for deciphering our in vivo data obtained in the multiple sclerosis (MS) murine model of experimental autoimmune encephalomyelitis (EAE). In EAE mice, we found a marked and persistent upregulation of GPR17 in the OPCs accumulating at demyelinating lesions; in a similar way, in autoptic samples from MS patients, many GPR17-positive activated cells accumulated at the border of active lesions in parallel with a marked increase of SDF1 levels [Calderon et al 2006, J Neuroimmunology]. Conversely, no GPR17 upregulation was found in a model characterized by a much lower degree of inflammation, i.e., cuprizone-induced demyelination model. Thus, SDF1 may represent one of the key inflammatory factors triggering a persistent upregulation of GPR17 in both rodent EAE and human MS. We speculate that, as a results of chronic inflammation, SDF1 accumulating at demyelinating lesions markedly upregulates GPR17, thus preventing its physiological downregulation, that would, in turn, inhibit terminal OPC maturation to myelinating cells. These findings may have implications for the design of novel pharmacological approaches aimed at overcoming the re-myelination block typical of chronic demyelinating diseases.

Disclosures: **C. Parravicini:** None. **G.T. Coppolino:** None. **D. Lecca:** None. **M. Fumagalli:** None. **D. Marangon:** None. **S. Daniele:** None. **L. Palazzolo:** None. **C. Martini:** None. **E. Gianazza:** None. **M.L. Trincavelli:** None. **P. Zaratin:** None. **I. Eberini:** None. **M.P. Abbracchio:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

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Topic: B.13. Demyelinating Disorders

Support: Facial Pain Research Foundation

Title: Lipid abnormalities in myelinated nerves of Trembler J neuropathic mice

Authors: ***A. I. FETHIERE**, Y. ZHOU, E. SOTO, L. NOTTERPEK;
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Abstract: The Trembler J (TrJ) mouse harbors a non-conservative leucine to proline mutation in peripheral myelin protein 22 (PMP22) and models early onset dysmyelinating neuropathy. Peripheral nerves of young heterozygous animals contain abnormally thin myelin, which progress to demyelination in adulthood. Reduced expression and altered localization of Schwann cell and axonal proteins have been described in affected nerves, however much less is known about potential changes in lipid metabolism related molecules. In this study we investigated the expression and localization of cholesterol metabolism related proteins and the distribution of various myelin lipids. Sciatic nerves from young adult genotyped male and female mice were collected and used for morphological and biochemical studies. Western blot analysis of whole nerve lysates identified significant increases in the levels of apolipoprotein E (ApoE), ATP-binding cassette protein A1 (ABCA1), and lipoprotein receptor-related protein 1 (LRP1) in affected nerves, as compared to wild-type (WT). To investigate the cellular source for the elevated ApoE, we double immunolabeled nerve sections with anti-ApoE and anti-CD11b antibodies. In agreement with previous studies, there is an increase in the number of CD11b positive macrophages in affected nerves, as compared to age-matched WT, and these immune cells are reactive for ApoE. Microscopic analysis of polar and non-polar lipids using Nile Red, a lipophilic stain, on nerve sections revealed substantial decrease in overall lipid-associated signal, which can be attributed to the hypomyelinated state of the TrJ nerves. In addition, we observed clumped, irregular distribution of lipids in the neuropathic nerves. Utilizing filipin, a fluorescent dye that specifically binds to cholesterol, we observed irregular and clustered cholesterol distribution on affected TrJ nerves. Furthermore, filipin staining on TrJ Schwann cells shows

limited transport of cholesterol to the plasma membrane with the majority localized around the perinuclear area, as compared to WT. Together, the upregulation of proteins involved in lipid trafficking and the irregular lipid distribution in TrJ nerves suggest that the intracellular aggregation and altered trafficking of the mutant PMP22 impacts lipid homeostasis.

Disclosures: **A.I. Fethiere:** None. **Y. Zhou:** None. **E. Soto:** None. **L. Notterpek:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

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Topic: B.13. Demyelinating Disorders

Support: Multiple Sclerosis Society of Canada Research Grant EGID 1762

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Title: A model of failed remyelination demonstrates a crucial role of new oligodendrocytes on motor recovery and preservation of axons.

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Abstract: Demyelinating inflammatory lesions are the hallmark of multiple sclerosis (MS). Remyelination can occur but this process is often incomplete resulting in the chronic demyelination of axons. The failure to remyelinate, has been correlated with increased axonal damage which is thought to underlie progressive disability. In contrast to MS, remyelination in rodents following chemical demyelination is typically rapid and efficient. Therefore, there is need of an animal model that selectively inhibits oligodendrocyte maturation while leaving other cellular responses to demyelination unaltered. Such a model would allow for the exploration of the role of remyelination in axonal health, the mechanisms by which chronically demyelinated axons are lost, and for testing the effectiveness neuroprotective therapies on preserving demyelinated axons. Remyelination requires the recruitment and maturation of oligodendrocyte precursor cells (OPCs) into new oligodendrocytes, and selectively blocking this process would

halt remyelination. Previously, we found the inducible deletion of a crucial transcription factor for myelination from OPCs, myelin regulatory factor (Myrf), inhibits new oligodendrocyte production and impairs subsequent remyelination. These mice, combined with cuprizone/rapamycin demyelination, should permit the modelling of impaired remyelination *in vivo* without altering inflammation or astrogliosis directly. $Myrf^{fl/fl}$ PDGFRa CreER (Myrf iCKO) and $Myrf^{fl/fl}$ controls were injected daily with rapamycin (10mg/kg) and fed a diet containing 0.3% cuprizone for six weeks to induce near complete demyelination of the medial corpus callosum, then returned to a normal diet to permit remyelination. In the fifth week of cuprizone intoxication, the mice were injected with tamoxifen to delete Myrf from OPCs. In contrast to tamoxifen-treated control mice, Myrf iCKO mice did not demonstrate accumulation of new oligodendrocytes when returned to a normal diet. Electron microscopy and immunohistochemical stains reveal little evidence of remyelination. Myrf iCKO mice exhibited reduced motor coordination as assessed on the Rotarod following cuprizone demyelination. The number of SMI31+ axons was decreased in the corpus callosum of Myrf iCKO mice, suggesting new oligodendrocytes are crucial for preservation of phosphorylated neurofilament positive axons. The density of microglia and astrocyte reactivity was examined, as was the number of SATB2+ neurons. Myrf iCKO mice combined with cuprizone demyelination will offer new insights into the mechanisms and consequences of failed remyelination on axonal preservation and functional outcomes.

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Poster

511. Demyelinating Disorders and Their Mechanisms

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Topic: B.13. Demyelinating Disorders

Support: NIH, NINDS Grant NS43783

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Title: Axon initial segment changes in a potential new mouse model of Multiple Sclerosis

Authors: *D. Z. RADECKI[1], C. R. RICHARDSON[1], A. BROWN[6,2], S. PERRINE[3], A. GOW[1,4,5];
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Abstract: Multiple Sclerosis (MS) is a demyelinating disorder of the CNS for which secondary gray matter (GM) damage, including lesions and progressive neurodegeneration, is reemerging as an integral component of the pathophysiology. To investigate this secondary GM damage, our laboratory developed the novel *OBiden* (*OBi*) mouse model of MS pathology. In *OBi* mice, we generate primary, episodic metabolic stress in oligodendrocytes (OLs), whereby this primary white matter (WM) pathology damages neurons and causes GM changes reminiscent of MS. First gliosis, defined by significant activation of microglia, is observed in multiple WM tracts, likely arising as these cells remove cellular debris from stressed or dying OLs. Second, we observe similar overall degenerative changes in MS normal appearing GM (NAGM) and *OBi* cortex, such as increases in non-phosphorylated neurofilaments without changes to general neuronal or synaptic integrity markers. We also find changes to the axon initial segment (AIS), the trigger zone for action potentials, which comprises shortened AISs in rostral entorhinal and perirhinal cortices of *OBi* mice. Also, we observe changes to the specific ion channel variant, potassium channel 7.2 (Kv7.2), a modulator of basal firing rate in neurons. However, the predominant change is seen in the CA1 layer of dorsal and ventral hippocampus, with a significant decrease in Kv7.2 signal on western blots and diminished fluorescence staining in the AIS. These changes appear to manifest as behavioral changes in *OBi* mice. Specifically, a learned helplessness (LH) depression-like endophenotype develops by 6 months of age and is maintained until at least 12 months, indicating dysfunction in hippocampal processing pathways. 12 month old *OBi* mice show deficits in two tests specific for short term memory function; novel object (N.O.) testing and a win-shift T-maze foraging paradigm. In N.O. testing, *OBi* mice fail to distinguish a novel object from a familiar object. The win-shift T-maze trains mice to alternate between goal arms of a standard T-maze to follow a food reward. Twelve month *OBi* mice show an inability to shift (alternate) arms on successive trials, again suggesting a deficit in working or short term memory. Together, this data demonstrate that *OBi* mice develop significant secondary GM changes from primary WM pathology in OLs with parallels to MS molecular and cognitive pathology.

Disclosures: D.Z. Radecki: None. **C.R. Richardson:** None. **A. Brown:** None. **S. Perrine:** None. **A. Gow:** None.

Poster

511. Demyelinating Disorders and Their Mechanisms

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The Legacy of Angels Grant

Title: Behavioral recovery due to knock out of alpha-synuclein in a mouse model of Krabbe disease

Authors: *M. S. MARSHALL[1], Y. ISSA[2], V. ELACKATTU[2], B. JAKUBAUSKAS[2], F. CARDOZO-PELAEZ[3], E. R. BONGARZONE[2];
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Abstract: Infantile Krabbe disease is a devastating genetic disorder, which causes progressive demyelination of the central and peripheral nervous system, neurosensory deficits, muscle atrophy, and early death. Krabbe disease is due to loss-of-function mutations in the gene encoding for the lysosomal enzyme Galactosylceramidase (GALC). This results in the toxic accumulation of one of its lipid substrates, galactosyl-sphingosine (or psychosine).
We have recently identified the presence of psychosine-induced intraneuronal aggregates of mis-folded alpha-synuclein (α-syn) in the brains of both the Twitcher (TWI) mouse, the natural murine model of KD, and human KD patients. This finding is remarkable because KD patients show movement symptoms similar to many of the hallmark symptoms in another adult movement disorder and alpha-synucleinopathy, Parkinson's disease (PD). Additionally, the accumulation of these aggregates within the substantia nigra of the TWI prompted us to examine the levels of dopamine (DA) in the TWI, which we found to be significantly reduced in the caudate at postnatal ages 15 and 30 days. This suggests that aggregations of alpha-synuclein may be a contributing factor to the pathology of KD.
Alpha-synuclein is known to have influences over the production of dopamine and its synaptic release. To isolate the role alpha-synuclein aggregates play in KD, we created a new mouse model that crosses the twitcher with a knock out of *SNCA*, the gene for alpha-synuclein. This TWI/SNCA-KO animal shows a significant increase in lifespan, body weight, grip strength, and

nesting ability when compared to the twitcher. These mice also displayed an improvement in locomotion and gait. These results suggest that the effects of alpha-synuclein compound the pathophysiology of KD in addition to the already toxic effects caused directly by psychosine. These results provide a new therapeutic target for the treatment of KD and potentially open a new model for studying the detrimental effects of alpha-synuclein aggregation.

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Poster

512. Brain Wellness

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 512.01/V18

Topic: C.01. Brain Wellness and Aging

Title: Loss of the *Drosophila* khc-73, kinesin-3 family member, causes age-dependent neurodegeneration

Authors: *E. MAKSOUD, E. LIAO, P. HAGHIGHI;
The Buck Inst. For Res. On Aging, Novato, CA

Abstract: Neurodegenerative diseases are currently incurable and are characterized by a progressive loss of neuronal cells. Dysregulation of endosomal dynamics has been suggested as one of the common causes of a number of degenerative disorders such as Parkinson's disease, amyotrophic lateral sclerosis and hereditary spastic paraplegia. Tight regulation of endosomal trafficking is essential for appropriate signaling at the synapse and efficient transport of cellular cargoes from the soma to the processes and vice versa. We have recently generated mutations in the fly *Khc-73* gene, a member of the kinesin superfamily and a homolog of the human *Kif13B*, which leads to defects in the retrograde routing of late endosomes from synaptic terminals to the retrograde pathway in motoneurons. *Khc-73* loss of function mutant flies appear normal in the first few days following hatching, but soon develop climbing and flying defects. One of the hallmarks of neurodegeneration is the detection of lesions in the brain that appear as vacuoles in histological sections. Compared to age-matched control flies, *Khc-73* mutant flies showed strikingly increased numbers of vacuoles and larger vacuoles in histological brain sections. This severe degeneration was fully reversed in *Khc-73* mutant flies when we genetically introduced a genomic copy of the *Khc-73* gene. In order to determine the tissue requirement for *Khc-73*, we took advantage of a transgenic RNA interference approach, and showed that knock down of *Khc-73* in neurons, but not in glial cells, was capable of recapitulating the phenotype in *Khc-73* loss

of function mutants. Finally, to rule out a developmental role for *Khc-73* we verified the temporal requirement of *Khc-73* by knocking it down only after birth in adult flies. Temporally restricted knock down of *Khc-73* in adult neurons also led to a severe neurodegeneration reminiscent of the loss-of-function phenotype. These results indicated that *Khc-73*'s function is required in neurons to ensure their healthy aging and to prevent early onset neurodegeneration in adult flies. Based on the role of *Khc-73* as a regulator of endosomal trafficking, our findings provide strong evidence that disruptions in endosomal trafficking during aging may result in accumulative damage in neurons and ultimately trigger neurodegeneration.

Disclosures: E. Maksoud: None. **E. Liao:** None. **P. Haghighi:** None.

Poster

512. Brain Wellness

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 512.02/W1

Topic: C.01. Brain Wellness and Aging

Support: University of Sri Jayewardenepura

Title: Banking the brain & blood: A Sri Lankan emerging era of collaborative research; what East can offer the West

Authors: *R. DE SILVA;
Univ. of Sri Jayewardenepura, Nugegoda, Sri Lanka

Abstract: Introduction: Variations in the genetic dispositions, influenced by environmental factors including diet, natural products, lifestyle factors, ethnicity and culture have profound influence in: differential responses in clinical settings, neurobiologic biomarkers that may be associated with neurological diseases. The human brain bank and DNA repository established in Sri Lanka, is one of the largest bio-banks in the Indian subcontinent that could facilitate as a cornerstone in translational neuroscience. **Objectives**: To facilitate the access of biological resources of South Asia and support high-quality multidisciplinary research collaborations in neuroscience understanding the diversity of neurological disorders, bridging the East and the West. **Method:** Anatomico-pathological studies performed in cerebral arteries of 447adult and 34 fetal postmortem brains and gene expression studies in 6 cerebral arteries. Age-related cytoskeletal pathologies in 76 aging and diseased human brains using histopathological/ immunohistochemical techniques for tau and β-amyloid biomarkers, and vascular genetic variants: apolipoprotein E, angiotensin converting enzyme, methylenetetrahydrofolatereductase (MTHFR C677T) and Factor V Leiden (FVL G1691A). A bio-repository of DNA bank

established with socio-demographic and clinical data of over 2,000 patients with; [Stroke n=1200, Parkinson's Disease (PD) n=250, Duchenne Muscular Dystrophy (DMD) n=130, Spinal Muscular Atrophy (SMA) n= 40, Huntington's Disease (HD) n=41, Spinocerebellar Ataxia (SCA) n= 49] and controls n=500. We possess unique samples; genetically confirmed monozygotic DMD twins (7th reported worldwide), PD patients with heavy metal & pesticide exposure and three villages located in the southern part of Sri Lanka with SCA type 1. **Results:** Burden of Alzheimer's disease related pathologies in aging brains did not show a significant difference between the East and West, significant anatomical variations in the circle of Willis and vascular genetic variants were seen among intra and inter ethnic groups, will be discussing how the bio-bank could offer novel insights in determining the ateiology of neurodegenerative and neuromuscular disorders, stroke and suicide. **Conclusion:** Anatomical, pathological and genetic variation between East and West is possibly due to genetic, racial, regional, environmental and dietary factors or a combination of any of the above, warrants further multidisciplinary collaborative studies between West and East, culture unique to East may lead to the development of preventive therapeutics, nutraceuticals that could promote healthy brain aging.

Disclosures: R. De Silva: None.

Poster

512. Brain Wellness

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Program#/Poster#: 512.03/W2

Topic: C.01. Brain Wellness and Aging

Support: NIH GRANT GM008395

Title: Do over-the-counter brain supplements alleviate neurodysfunction? An extensive trial using the spastic Han Wistar rat, a model of ataxia

Authors: *R. A. HERNANDEZ[1], C. T. LAO[2], M. GARCIA[2], D. A. DICKEY[2], R. W. COHEN[2];
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Abstract: As an alternative to current drug, stem cell, and physical therapies, there are numerous forms of over-the-counter brain supplements. The projected benefits of using these brain supplements for numerous of brain injuries, including concussion, Parkinson's and Alzheimer's, have been proclaimed by their manufacturers to augment the effectiveness of pharmaceutical

drugs. The brain supplement we utilized in our project is made up of natural compounds such as niacin, acetyl- L-carnitine, phosphatidylserine, inositol, and choline that have been found to increase neuroprotection in many disorders. The goal of our project was to evaluate the effectiveness of an over-the-counter brain supplement on an animal model of cerebellar ataxia. The *spastic* Han-Wistar rat carries an autosomal gene mutation that induces various symptoms of ataxia, including forelimb tremor and hind limb rigidity. Studies in our laboratory have shown that the symptoms of the spastic Han Wistar rat originate from progressive Purkinje cell death. The symptoms of the spastic Han Wistar rat are evident beginning at age 25-30 days. Progression of the disorder eventually leads the rats to malnutrition and dehydration, due to aforementioned symptoms of ataxia that ultimately leads to their death at 65-70 days. Utilizing this animal model, we tested one over-the-counter brain elixir or vehicle on both normal and mutant rat groups (n=10 for treatment; equal mix of males and females). We randomly chose Han-Wister rats at 28 days old (control mutant and normal siblings) and began feeding elixir or vehicle (sucralose) daily (0.7mg/kg; per os). Rats were dosed daily until the mutant's date of death. We tested the decline in motor activity of the treated animals weekly using the rotorod and open field activity tests. Our results showed that mutants treated with a brain supplement had a slightly longer lifespan and had a slight incline for Rotorod test. For the Open field, the mutant groups showed signs of decline overtime. The effects of brain supplements on this animal model of ataxia show low impact on performance.

Disclosures: R.A. Hernandez: None. **C.T. Lao:** None. **M. Garcia:** None. **D.A. Dickey:** None. **R.W. Cohen:** None.

Poster

512. Brain Wellness

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Topic: C.01. Brain Wellness and Aging

Support: CONACyT scholarship 280276

Title: Effect of bexarotene on locomotor activity, learning and memory and dendritic morphology in old mice

Authors: *E. MONROY HERNÁNDEZ, ESQ[1], F. DE LA CRUZ[3], G. FLORES[2];
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Abstract: Bexarotene, a compound has been suggested as a neuroprotective in Alzheimer's disease. However its mechanism of action has not been fully described. Increasing age, leaves individuals at risk for neurodegenerative disorders like Alzheimer's disease. Although the vast majority of older adults do not develop dementia, usually some degree of cognitive change is experienced. The main objective of this work was to investigate changes in behavior and its correlation with neuronal morphology of the hippocampus, nucleus accumbens (NAcc) and prefrontal cortex (PFC) caused by the administration of bexarotene in old mice. For this, we administered intraperitoneally with repeat doses of bexarotene (0.0, 0.5, 2.5 and 5 mg/kg) for 21 days to 18 months male mice. A behavioral level, locomotor activity, and learning and memory was evaluated in the Morris water maze test. Neuronal morphology was evaluated using Cox Golgi staining. The results showed that mice administered with 5 mg/kg of bexarotene had increased locomotor activity, there is no significant differences in memory and learning test between animals administered with bexarotene; whereas in dendritic arborization, NAcc hypertrophy was found in the group with administration of 2.5 mg/kg of bexarotene, without differences in the density of spines compared with the control group and there were no change in hippocampal neuronal cytoarchitecture and PFC. The administration of bexarotene in senile mice did not offset the effects associated with aging in learning and in memory, although there was greater locomotor activity in the group administered with the dose of 5 mg/kg of bexarotene compared to the control group.

Disclosures: **E. Monroy Hernández:** None. **F. De la Cruz:** None. **G. Flores:** None.

Poster

512. Brain Wellness

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 512.05/W4

Topic: C.01. Brain Wellness and Aging

Support: Evelyn F. McKnight Brain Institute

Arizona Alzheimer's Consortium

Title: FTO gene and BMI interact to predict white matter integrity in late middle age and older adults

Authors: ***A. STICKEL**[1], K. WALTHER[3], M. HUENTELMAN[4], L. RYAN[2];
[1]Psychology, [2]Univ. of Arizona, Tucson, AZ; [3]Psychology and Neuropsychology, Ludwig Maximilians Univ., Munich, Germany; [4]Translational Genomic Res. Inst., Phoenix, AZ

Abstract: Unhealthy weight affects approximately two-thirds of Americans. In addition to lifestyle factors, genetics may contribute to unhealthy weight. The Fat Mass and Obesity (FTO) gene was one of the first to be linked to weight and is highly expressed in the brain. Both FTO and body weight have been associated with individual differences in brain structure and cognition (Ryan et al., 2014; Ho et al., 2010), but few studies have considered the combination of these two factors. The present study examined the combined contributions of FTO allele (rs1421085) and body mass index (BMI) on white matter integrity, controlling for age. Diffusion MRI scans (25 directions) were obtained from 128 adults (ages 52-92) on a 3T Skyra with a 32 channel head coil. In order of increasing risk for increased body weight, the study included FTO T-homozygotes (n = 43), T heterozygotes (n = 62), and C-homozygotes (n = 23). FTO groups were matched on gender, age, education, and percent hypertension. Phenotype was controlled by matching the groups on BMI (average BMI = 26, range = 18 to 45). Fractional anisotropy (FA), radial diffusivity (RD), and axial diffusivity (AD) values were calculated and compared on a voxel-by-voxel basis across segmented white matter maps in SPM8. Clusters were identified that showed a significant interaction between FTO and BMI, while controlling for age ($p < .05$, $k > 50$). While no interactions were observed for FA, an interaction was found for AD in the splenium: as BMI increased, AD decreased in the CC group but not the T carrier groups. Interactions were also observed for RD within the corticospinal tract, the splenium, and the anterior cingulate. Contrary to expectations, as BMI increased in each region, radial diffusivity decreased in the CCs, while T carrier groups showed no association between BMI and RD. The results suggest that CCs – individuals with the highest risk for obesity – have greater BMI-related changes in white matter diffusion relative to T carriers, beyond the changes associated with normal aging and BMI alone. The pattern of change is surprising. In most studies, damage to white matter is associated with increases in RD. Here, we find the opposite – increasing BMI is associated with decreased RD values. This finding might suggest enhanced myelin in these individuals (Song et al., 2003). Alternatively, at least one study (Elvsåhagen et al., 2015) has reported decreases in RD after sleep deprivation, and note that RD may be sensitive to other aspects of white matter including cell membrane permeability, axon diameter, tissue perfusion or glial processes. Our findings warrant future investigations into the pattern of white matter change associated with the FTO gene.

Disclosures: **A. Stickel:** None. **K. Walther:** None. **M. Huentelman:** None. **L. Ryan:** None.

Poster

512. Brain Wellness

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Program#/Poster#: 512.06/W5

Topic: C.01. Brain Wellness and Aging

Support: NSFGrant HRD-1463889

Title: The effects of long term treatment with withania somnifera on cognitive function

Authors: *S. ALI[1], A. LI[2], J. MARTINEZ[2], G.-A. LETICIA[2], I. VASQUEZ[2], A. MADIRA[2], E. CUELLAR[2], M. CANDELARIO[2], J. M. REYES-RUIZ[3], A. RUSSO-NEUSTADT[2];
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Abstract: *Withania somnifera* is an adaptogenic herb that has been traditionally used in the Indian Ayurvedic medical system for thousands of years. It has been found to have many beneficial properties, such as promoting relaxation, improving muscle strength, and exhibiting neuroprotective characteristics. Previous studies have suggested that *Withania somnifera* protects the brain against neurodegeneration. We hypothesize that, during the course of aging, long-term treatment with *Withania somnifera* will improve the performance of older rats in cognitive/behavioral tasks, and will also increase the expression of genes associated with learning and memory. The novel object recognition test, which assesses recognition memory through the identification by distinguishing a novel object from a familiar one, was employed in our studies. Briefly, Sprague-Dawley rats were placed in an open field arena in the presence of either two identical objects, or two different objects, one of which was novel after a 24-hour delay. Sprague-Dawley rats that were treated with *Withania somnifera* for fourteen months showed an increase in environmental engagement and higher discrimination index during the novel object recognition test. It was found that *Withania somnifera* treated rats had a mean discrimination index of 0.36 ± 0.05, while the mean discrimination index of the control group was 0.12 ± 0.03. A two-tailed unpaired *t*-test revealed a *p*-value of 0.0013. In order to assess the changes in gene expression during the course of the treatment, RTqPCR analysis of hippocampi from these animals were conducted. RNA was isolated from hippocampal using the RNeasy Kit (Qiagen) and cDNA was generated using the BioRad's iScript cDNA synthesis kit. The resulting cDNA was used as a template to perform qPCR with the SYBR Green mix from Bio-Rad and an iCycler real-time PCR machine. Our analysis revealed an elevation in the expression of glutamate AMPA receptor 2 (about twice the number of transcript vs. control, $p=0.015$, n=8). These results suggest that long term *Withania somnifera* treatment preserves recognition memory and environmental exploration/engagement, and may also enhance gene expression associated with excitation/inhibition balance in the CNS.

Disclosures: **S. Ali:** None. **A. Li:** None. **J. Martinez:** None. **G. Leticia:** None. **I. Vasquez:** None. **A. Madira:** None. **E. Cuellar:** None. **M. Candelario:** None. **J.M. Reyes-Ruiz:** None. **A. Russo-Neustadt:** None.

Poster

512. Brain Wellness

Location: Halls B-H

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Topic: C.01. Brain Wellness and Aging

Title: The c-terminal domain of the heavy chain of tetanus toxin effects in performance deficits and morphology in aged mice

Authors: *M. PACHECO-FLORES[1], R. VAZQUEZ-ROQUE[2], G. FLORES[2], J. C. PENAGOS-CORZO[4], J. AGUILERA[5], A. DÍAZ[3];
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Abstract: The deficits associated to aging involve a progressive decline in cognitive processes along with morphological alterations, in case of neurological and morphological deficits it has been showed that the prefrontal cortex and hippocampus are altered in aged mice, given the decline observed during aging of systems as learning and memory that correlate to these structures, several studies had target to shown if age-related changes could be offset by certain pharmacological compounds. It has been suggested that the C-terminal domain of tetanus toxin (Hc-TeTx) could be used as a neurotrophic factor involved in the activation of cascades responsible for trophic actions and neuroprotection that results in the amelioration of neurodegenerative processes. In this study we aimed to evaluate the effects of the (Hc-TeTx) treatment on behavioral and neuronal alterations. To assess the behavioral effects we used a paradigm that has been widely used to evaluate attentional and executive functions in rodents, the 5-choice serial reaction time task (5-CSRTT), for morphological evaluation we analyzed the changes in pyramidal neurons of the PFC (Layers III and V) and dorsal Hippocampus (DH), CA-1 and CA-3 regions of 18 month-old mice by using the Golgi-Cox method. In addition, mice were intraperitoneal injected with the recombinant Hc-TeTx protein (40µg/Kg, weekly, for two months). Furthermore, behavioral data showed that the Hc-TeTx group had an improvement in performance compared to the Control group, besides histological analysis showed a recovery effect induced by the Hc-TeTx in PFC and DH neurons. In conclusion, our results suggest that Hc-TeTx could serve as a potential therapy for age-related deficits considering the beneficial effects in behavioral deficits and morphological alterations.

Disclosures: M. Pacheco-Flores: None. **R. Vazquez-Roque:** None. **G. Flores:** None. **J.C. Penagos-Corzo:** None. **J. Aguilera:** None. **A. Díaz:** None.

Poster

512. Brain Wellness

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Program#/Poster#: 512.08/W7

Topic: C.01. Brain Wellness and Aging

Support: UH GEAR 14-15

Title: Interactive effects of weekly binge alcohol and exercise on the female brain

Authors: *R. WEST, J. I. WOODEN, E. A. BARTON, J. L. LEASURE;
Univ. of Houston, Houston, TX

Abstract: Mounting evidence substantiates a positive association between physical activity and alcohol consumption, with alcohol drinkers being more physically active. The interactive effects of binge alcohol and exercise on the brain therefore warrant investigation. We focused on the female brain, as binge drinking is a common alcohol use disorder (AUD) among American women and girls. According to the Centers for Disease Control, 1 in 8 report binge drinking three times a month (6 drinks per binge). We have previously shown that a single 4-day binge results in a 10-15% decrease in granule neurons in the hippocampal dentate gyrus (DG) in female rats. We have also shown that 2-4 weeks of post-binge exercise restores the damaged DG. In the present study, we hypothesized that weekly binge alcohol would cause a significant loss of DG granule neurons, and that weekly exercise would prevent this. Female adult Long-Evans rats (n=45) were randomly assigned to either control or alcohol groups and given a once-weekly alcohol (5 g/kg) or isocaloric control dose via intragastric gavage for 11 weeks. Following the alcohol dose, rats either remained sedentary or were given access to exercise wheels (2 hours/3 consecutive days). Behavioral intoxication and blood alcohol content were measured weekly and did not differ between binged groups. All rats were behaviorally tested periodically throughout the experiment. Exercised rats, regardless of binge exposure, were more exploratory in the open field and performed better on the rotarod in comparison to sedentary rats. We found no difference between groups on a water maze task, however sedentary binged rats had impaired object recognition compared to all other groups. Brain tissue was stained for the neuronal marker, NeuN, and remaining granule cells in the DG were counted using unbiased stereology. Rats that remained sedentary had a 19% reduction in remaining granule neurons compared with sedentary controls, and exercise reversed this effect. Our results indicate that weekly binge alcohol causes detectable hippocampal damage and cognitive impairment, and that exercise counteracts these effects. Our next step is to determine whether weekly binge alcohol influences exercise-driven neuroplasticity.

Disclosures: **R. West:** None. **J.I. Wooden:** None. **E.A. Barton:** None. **J.L. Leasure:** None.

Poster

512. Brain Wellness

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Topic: C.01. Brain Wellness and Aging

Support: NIH Grant U01 AG024904

Title: Ventriculomegaly and periventricular pathology correlate with cognitive impairment in the aging brain

Authors: *K. TODD[1], E. NORTON[1], T. BRIGHTON[1], S. SCHICK[1], S. RESNICK[2], W. ELKINS[2], J. TRONCOSO[3], O. PLETNIKOVA[3], J. CONOVER[1];
[1]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT; [2]Natl. Inst. on Aging, Natl. Inst. of Hlth., Baltimore, MD; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Expansion of the brain's fluid-filled ventricles, termed ventriculomegaly, is a condition commonly found in the aging brain. We have previously shown that expansion of the lateral ventricles is linked to ventricle surface gliosis in which areas of the ciliated ependymal cell monolayer that lines the ventricles are replaced with dense astrocytic patches. Loss of ependymal cells would compromise trans-ependymal bulk flow mechanisms required for clearance of harmful toxins and metabolites from the parenchyma. However, the interplay between age-related ventricle expansion and decline in ependymal integrity and its effect on periventricular fluid homeostasis, pathological protein accumulation and cognitive impairment has not been examined. In collaboration with the Baltimore Longitudinal Study of Aging and Alzheimer's Disease Neuroimaging Initiative, we analyzed longitudinal structural MRI scans to map spatiotemporally the progression of ventricle expansion in healthy aging individuals and those with varying degrees of cognitive impairment. Accompanying subject-matched FLAIR-(fluid-attenuated inversion recovery) MRI scans and biospecimens were used to investigate the relationship between periventricular white matter hyperintensities and loss of ependymal layer functions in areas of ventricle expansion. We found that the trajectory of ventricle expansion correlated with degree of cognitive impairment in both speed and severity, and confirmed that in areas showing longitudinal expansion ependymal cells were replaced with regional gliosis. The relative size of periventricular white matter hyperintensities increased with worsening cognitive performance. This was accompanied by accumulation of protein aggregates in periventricular tissue, further suggesting impaired clearance mechanisms in these regions. These findings provide valuable and highly relevant information for the development of improved diagnostic tools and treatments for the healthy and cognitively impaired aging population.

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Poster

512. Brain Wellness

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: C.01. Brain Wellness and Aging

Support: VCU President's Research Quest Fund

Title: Making American Football Safer: changing the NFL's policy on the type of helmets allowed on the playing field

Authors: ***R. J. COLELLO**[1], I. COLELLO[3], M. GRASSO[2], O. KEMP[2], S. MUFFLY[2], K. OIU[2], A. SCHLOE[2];
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Abstract: Helmet-to-helmet collisions are considered one of the primary means by which concussions occur in American Football. With over 1,500,000 people playing football nationwide, helmet-to-helmet contact accounts for a significant proportion of the over 100,000 concussions that occur yearly. Not surprisingly, this represents a health concern of such magnitude that many have questioned whether the game can survive. In an effort to evaluate helmet safety, the STAR System was developed in 2011 that rated helmets based on their ability to reduce g forces experienced by the head across a range of impact forces measured on the field. Using a helmet drop assay to measure linear forces, helmets were rated 1-5 Stars, with a 5-Star rating being the best available helmets and a 1-Star the worst. Although this was a major step in making the game safer, litigation concerns made the NFL, in 2014, adopt the position of allowing players to wear any helmet provided it is, "of a suitably protective nature" and "designed and produced by a professional manufacturer". This led us to ask: 1.) what helmets do NFL players wear and, 2.) does the current NFL helmet policy make the game safer? To address this, we identified the specific helmets worn by 1000 NFL players on Week 13 of the 2015 season. Using stop motion footage for each of the16 games played and helmet air vent patterns to identify helmets worn, we found that players wore a wide range of Star Rated Helmets, from the 5-Star Riddell Revolution Speed (44%) and Schutt Air XP Pro (33%) to the 3-Star Riddell Revolution (6%) and 1-Star Riddell VSR4 (worn by Tom Brady, Drew Brees and 2% of players). Using a helmet-to-helmet impactor system, we next sought to determine the g forces

experienced by the head when helmets of varying Star rating collided through the range of impact forces recorded on the field. We found that, when two 5-Star helmets collided, the g forces experienced by the head were roughly 25% less than that observed for two 3-Star helmets colliding and roughly 50% less than that observed for two 1-Star helmets colliding. Surprisingly, when a 5-Star helmet collided into a 1-Star helmet, the 5-Star helmet's ability to protect the head was significantly reduced, with g forces experienced by the head in the 5-Star helmet increasing by an average of 40% at all impact forces tested. Together, these results suggest that the NFL policy of allowing players to wear any helmet put players at increased risk of receiving a concussion during helmet-to-helmet collisions. Thus, the simplest and most straightforward way to reduce concussions in American Football is to mandate that players only wear helmets that receive the highest safety rating.

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Poster

512. Brain Wellness

Location: Halls B-H

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Program#/Poster#: 512.11/W9

Topic: C.01. Brain Wellness and Aging

Title: Nuts and brain: Walnuts, pecans, and cashews increase power spectral density (μV^2) of EEG gamma wave band frequency (31- 40 Hz) relative to nut antioxidant concentration - beneficial for enhancement of recall, memory and brain wellness.

Authors: *L. S. BERK[1,2,3], E. LOHMAN[2], G. BAINS[1], N. DAHER[4], J. LEE[2], J. BRADBURN[5], R. MOLINA[7], S. JUNEJA[2], R. MOHITE[2], N. VIJAYAN[2], S. MORE[2], P. DESAI[2], D. MALI[6], S. SHAH[2];
[1]Allied Hlth. Studies, [2]Physical Therapy, [3]Sch. Med., [4]Res. & Statistics, [5]Clin. Lab. Sci., [6]Loma Linda Univ., Loma Linda, CA; [7]Global Hlth. & Digital Educ, Sch. Publ. Hlth, Loma Linda Univ., Loma Linda, CA

Abstract: Nuts are a major source of flavonoids. They are potent antioxidants with known mechanisms that provide cardioprotective, anticarcinogenic, and anti-inflammatory properties. Studies have shown that absorbed flavonoids penetrate and accumulate in brain hippocampal regions involved in learning and memory. Neurobiological correlates of flavonoids cascade an expression of neuroprotective and neuromodulatory proteins that promote neurogenesis, blood-flow improvement, and angiogenesis supporting brain wellness. However, the correlates of neuroelectric activities that are associated with nut flavanoid effects on neurocognition, neuronal

synchronization, memory, recall, mood and behavior are not well known. Purpose: Provide evidence of a relationship between antioxidant concentration in nuts and electroencephalography (EEG) brain state frequency modulation, specifically gamma wave band frequency 31-40 Hz (γBA). Methods: A pilot study was conducted using walnuts, pecans, and cashews. EEG Power Spectral Density μV^2 (PSD) was acquired during a sequence of enhancing sensory awareness tasks ranging from cognition of past experience, visualization, olfaction, taste, and finally consumption of nuts. EEG wave band activity was recorded from 9 cerebral cortical scalp regions F3, Fz, F4, C3, Cz, C4, P3, Pz and P4 using the FDA approved EEG B-Alert 10X System™, Carlsbad, CA. Second by second 9 bandwidths (BW) were recorded through the study. The PSD BW data were referenced to eyes closed baseline task, and then Z-scored. Results: Z-scores were graphed and analyzed for each task along with BW across 0-40 Hz. The overall respective BW were collapsed across all 9 EEG channels. γBA for PSD was greatest in all 9 brain regions for all sensory tasks ($p<0.01$). The most profound observation was the composite of sensory tasks for γBA. It was observed that γBA was the highest for walnuts (PSD 4.5), with an antioxidant concentration of 2772 μmoles; followed by pecans (PSD 3.0), with an antioxidant concentration of 1743 μmoles; and next for cashews (PSD 1.4), with an antioxidant concentration 48 μmoles. Conclusion: This pilot study provides evidence that EEG γBA can be initiated by cognitive, sensory and consumption of nuts. It appears that the PSD γBA intensity is associated with anti-oxidant concentration. We propose this protocol as a potential assessment tool in determining the efficacy of nuts dose response modulation of PSD γBA 31-40 Hz for support of brain wellness. Further research is needed to support and expand these important preliminary findings.

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Poster

512. Brain Wellness

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Program#/Poster#: 512.12/W10

Topic: C.01. Brain Wellness and Aging

Title: The role of CD36 in atherosclerosis and vascular cognitive impairment

Authors: ***K. CHO**[1], Y. KIM[2], J. KIM[1], G. KIM[1];
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Abstract: CD36, a platelet receptor bound thrombospondin, contributes to normal and also various pathologic processes such as apoptotic cell death, fatty acid transport, atherosclerosis, and Alzheimer disease (AD) by initiating different signaling responses. The multi-ligand recognition of CD36 can allow exerting several functions depending on the different cell type. In Alzheimer brain, CD36 has been implicated to involve in neuronal degeneration and interruption of CD36 signaling pathways by blocking the recruitment of microglia to amyloid deposits. Here this study primarily (1) investigates the expression of peripheral CD36 and brain CD36 to age with cerebrovascular damge; (2) also examine the role of CD36 that works in initiating autophagy which may relate to atherothrombus forming and progessing; (3) Furtherly we examine how the blockage of MAPKinases involves and regulates the CD36 of endothelial and microglial cells with age. By the physiological aging process and vessel damage, CD36 expression in the human serum was increased contrast young and middle-aged group and also CD36 expression level was higher in aged group with cerebrovascular damage than without damaget. And also the aged who showed higher level of CD36 were relatively reduced cognitive function (MMSE, CDR). To further investigate the role of peripheral blood CD36 and brain CD36, we assessed the expression level in endothelial CD36 and microglial CD36. By inhibiting MAPKinases, we evaluate the changing downstream signal regulation and autophagy and apoptotic cell death.This study demonstrates that CD36 can promote MAPKinse signaling that may drive authophagic and apoptotic cell death in endothelial cells and microglial cells. This study suggest that increased CD36 with age leading age-related diseases could be prevented the consequences by not only treating antagonist (up-stream) but also blocking MAPKinase signals (down-stream).

Disclosures: **K. Cho:** None. **Y. Kim:** None. **J. Kim:** None. **G. Kim:** None.

Poster

512. Brain Wellness

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Topic: C.01. Brain Wellness and Aging

Title: Gender-specific associations of cardiovascular risk with brain structure across the lifespan

Authors: *A. ISENBERG, J. PA;
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Abstract: Declines in brain health with age are a major public health issue and may occur in men and women differently. In addition to the devastating emotional and physical caregiving burden placed on families worldwide, the cost of caring for the elderly is causing ever increasing

societal and economic burden. Despite great advances in investigating factors associated with cerebrovascular disease and dementia, progress towards effective prevention and treatment has been discouraging. Modifiable lifestyle interventions are promising for decreasing the risk associated with declines in brain health, with the potential for delaying disease onset and slowing decline in cognition. Known contributions of vascular risk factors associated with Alzheimer's disease (AD) may differ significantly by gender, but these effects have not been studied well in the context of cardiovascular disease. In the present study we hypothesized that cardiovascular risk factors may impact brain structures differently in men and women. Cardiovascular risk was assessed using the Framingham Risk score for cardiovascular disease (CVD) in the adult population of the publicly available Rockland NKI dataset (Ages 22-85 years old, N=426). CVD risk scores were calculated with a model based on the following factors: age, systolic blood pressure, treatment of hypertension, body mass index, diabetes, and smoking. Analyses were performed on the subset of these data with available MRIs (N=335) to assess the correspondence of cardiovascular risk with brain structure across the lifespan. Increased risk of cardiovascular disease across the lifespan was associated with smaller gray matter volume in men, particularly in superior frontal cortex, the hippocampus, parahippocampal gyrus, and the amygdala. In contrast, greater risk of cardiovascular disease in women was only associated with smaller thalamic grey matter volume. These data suggest gender may be a significant factor in how cardiovascular risk is associated with brain volume over the lifespan.

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Poster

512. Brain Wellness

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Topic: C.01. Brain Wellness and Aging

Support: NIH GRANT K01-DA034728

Title: The effects of heavy cannabis use during adolescence on hippocampal sub-region morphology in an aging population

Authors: Z. MAHMOOD[1], A. M. KARACOZOFF[1], T. M. HARRISON[2], S. Y. BOOKHEIMER[1], *A. C. BURGGREN[3];
[1]Psychiatry and Biobehavioral Sci., [2]Neurosci. Interdepartmental Ph.D. Program, UCLA, Los Angeles, CA; [3]Semel Neuropsychiatric Inst., Univ. California @ Los Angeles, Los Angeles, CA

Abstract: Adolescents and young adults of the 1960's and 70's, a time during which cannabis (CB) use expanded rapidly, are now entering their senior years when age-related cognitive decline may begin. There is growing evidence of the adverse effects of CB use on cognition, brain structure and function, specifically with earlier exposure during adolescence (1), but the long-term effects of heavy adolescent CB use in early life and its interaction with brain aging are not well understood. The current study used retrospective assessment in older adults to investigate CB use during adolescence and age-related changes in hippocampal complex (HC) morphology in late-life. We enrolled 22 subjects, 10 participants who used CB > 20x/month for at least a year during adolescence ('CB+'; M_{age} = 62 years old) and 12 subjects who did not report CB use during adolescence ('CB-'; M_{age} = 65 years old) based on self-report on the Marijuana Smoking History questionnaire (2) and an extensive Drug Use History Battery. No participants used CB at the time of testing, as verified by a urine test on the day of testing. High resolution hippocampal structural scans (TR:3s; TE:41ms; FOV=20mm, 512x512, 3mm thick, 0mm spacing) were acquired and a cortical unfolding method was used to measure thickness of HC subregions (3-4). Light cigarette smoking and alcohol use was allowed and matched across groups (5). Participants in the CB+ group had thinner cortex within Cornu Ammonis (CA) fields 2,3 and the dentate gyrus (11.9% thinner, p=0.004), CA1 (9.8% thinner, p=0.009), entorhinal cortex (7.9% thinner, p=0.0003) and subiculum (7.2% thinner, p=0.008). Across the whole HC, subregions averaged together showed 8.9% thinner gray matter in CB+ compared to CB- (p=0.005). The results suggest that chronic use of CB in adolescence has long-lasting effects on HC structure, which may underlie and exacerbate age-related cognitive decline. Our findings suggest that CB use has a neurotoxic effect on the adolescent brain that persists well into adulthood. These findings, when expanded to a larger sample size, may help to identify persons more likely to decline than their age-matched counterparts and suggest early life CB use may have long-lasting effects on brain morphology in the HC.
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Poster

512. Brain Wellness

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Topic: C.01. Brain Wellness and Aging

Support: UK Medical Research Council: G1001354

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Title: UCP2 haplotype establishes a novel genetic link between mitochondria and mood disorders

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Abstract: Objective: Uncoupling protein (UCP) 2 is a proton carrier found in the inner mitochondrial membrane where it plays a role in regulating the membrane potential. UCP2 is expressed in neurons in various brain areas and plays a role in functions from lipid/ glucose metabolism to calcium signaling and the generation of reactive oxygen species. In humans several polymorphisms of UCP2 exist and they have been linked to body mass index variability and obesity. However, there are no studies to date showing links between UCP2 polymorphisms and the human brain.
Methods: Data from the Whitehall II imaging sub-study was investigated. Participants are former civil servants who are mostly male and now aged between 60 and 85 years. They were genotyped for two polymorphisms of the UCP2 gene, -866G>A (rs659366) and Ala55Val (rs660339). There is linkage disequilibrium between these polymorphisms and -866G is strongly linked to Ala55. Therefore, we investigated three different UCP2 haplotypes: homozygotes for -866G / Ala55 (called haplotype1 (HT1)), homozygotes for -866A / 55Val (HT3), and heterozygotes (HT2). We investigated their risk of developing neurodegenerative or psychiatric disorders. In addition, we investigated magnetic resonance imaging (MRI) data to determine the effect of UCP2 haplotypes on grey matter structure and functional brain connectivity using resting-state functional MRI.
Results: There was a significant UCP2 haplotype effect on the occurrence of mood disorders. There was a gene-dose effect with the highest occurrence of mood disorders in the HT3 group and a stepwise decrease to HT2 and HT1. The neuroimaging data showed a trend to decreased size of the thalamus for HT3 compared with the other two haplotypes. Additionally, this group showed significantly decreased functional connectivity of the thalamus with several cortical areas compared with the two other groups.
Conclusions: This is the first study to report an association between UCP2 haplotype and mood disorders in humans. The polymorphisms investigated here affect levels of UCP2 mRNA expression and thus mitochondrial function *in vitro*. While some evidence points towards the role of mitochondria in psychiatric disorders, we show a more direct genetic link between mitochondrial function and the occurrence of mood disorders. UCP2 haplotype effects on size and connectivity of the thalamus indicate that this region might be particularly susceptible to differences in mitochondrial function. As the thalamus is an integral part of the cortico-limbic

mood regulating circuit, differences in thalamic size and connectivity might lead to higher risk of mood disorders in the HT3 group.

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Poster

512. Brain Wellness

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 512.16/X2

Topic: C.01. Brain Wellness and Aging

Support: Boehringer-Ingelheim Alkmaar The Netherlands

Title: How the emotional motor system controls the pelvic organs

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Abstract: The brain has two goals, survival of the individual and survival of the species. Its most important tool is its motor system, which consists of the voluntary and the emotional motor system. The pelvic organs are very important parts of the body systems, because the bladder and rectum play a crucial role in micturition and defecation, while the prostate, testicles, vagina and uterus are the organs that take care of survival of the species.
The emotional motor system controls these pelvic organs, which are innervated by the sacral parasympathetic motoneurons. These motoneurons, in turn, are controlled by a specific group of neurons in the pontine brainstem, the pelvic organs stimulating center (POSC). Via long descending pathways the POSC generates micturition, defecation, parturition and sexual activities by stimulating different groups of sacral parasympathetic motoneurons. In turn, the POSC is driven by the periaqueductal gray (PAG), and pre-optic area (POA), which receive, via long ascending pathways from the sacral cord, precise information regarding the situation in all pelvic organs. The PAG and POA also receive instructions from higher brain regions as amygdala, and bed nucleus of the stria terminalis.
In humans the most important brain region having access to the PAG is the medial orbitofrontal cortex. This part of the prefrontal cortex receives information from many other cortical regions regarding the situation of the individual. Is the situation appropriate for micturition, defecation, sexual activities, or parturition to take place? If not, the orbito-frontal cortex will send a message to the pre-optic area and PAG not to activate the POSC in order to activate one of the pelvic

organs.
It has been shown that cerebral infarctions can cause urge-incontinence. In all likelihood the cause of urge-incontinence, in the very many elderly suffering from this disease, leading to healthcare costs of $85 billion a year, is interruption of the pathway of the orbitofrontal cortex to the PAG. The reason is the many small infarctions in the brains of these elderly.
In women suffering from hypoactive sexual desire disorder (HSDD), but not in healthy women, the medial orbitofrontal cortex is de-activated during watching erotic movies. This de-activation causes major problems, because the pre-optic area and the PAG are not activated in order to generate vaginal vasocongestion and lubrication, leading to decreased sexual behavior in general.
Conclusion, the orbitofrontal-POA-PAG-POSC-sacral parasympathetic motoneuronal pathway plays a crucial role in survival of the individual and species and problems in this system causes great problems in very many humans.

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Poster

512. Brain Wellness

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Program#/Poster#: 512.17/X3

Topic: C.01. Brain Wellness and Aging

Title: Determination of neurite isolation method and identification of neurite specific proteins in hydrogen peroxide-treated N1E-115 cells

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Abstract: Reactive oxygen species (ROS) increase the risks of several diseases, such as Alzheimer's disease (AD), cancer and diabetes. In these severe ROS-related diseases, we are focusing on the relationship between ROS-related brain dysfunction and aging. Accumulation of oxidative damage in the brain, the neurotransmission systems gradually attenuate. Although the brain is susceptible to damage by ROS, it is difficult to recover the neuronal network after induction of cell death. In order to prevent ROS-related neuronal cell death, we are trying to find an early sign of neurons before induction of cell death. Previously, we found that treatment with a low concentration of hydrogen peroxide induced axonal degeneration in cultured cells, and co-treatment with vitamin E protected its deterioration. Furthermore, collapsing response mediator protein (CRMP)-2, which one kind of microtubule-related protein phosphorylated in normal aged-mice brain. In this study, we tried to determine other neurite specific proteins in hydrogen

peroxide-treated N1E-115 cells using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) technique. Before start of this study, we tried to separate neurite and cell soma using cell-culture insert system. A few days after the culture cells using this system, we collected neurite, and checked tubulin and actin protein expressions by western blotting. Histone which is nucleus specific protein was not detected at all. Next, these proteins were purified and were labeled cyclopaedically by iTRAQ. At the result of LC-MS/MS, several protein expressions significantly increased in the isolated neurite from hydrogen peroxide-treated cells. Some proteins which we identified have already reported to be associated with neurodegeneration disorders. These results show the possibility that ROS-derived neurite degeneration is induced in the early stage of neurodegeneration disorders. However, it is difficult to obtain the reproducibility, we are continuing to study the identification of proteins and the elucidation of its function.

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Poster

513. Alzheimer's Disease: Therapeutics

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Program#/Poster#: 513.01/X4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R01AG037637-01

Title: Maternal choline supplementation as a preventive therapeutic strategy for reducing AD-like pathology

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Abstract: Over the next few decades, the advancing age of the global population will dramatically increase the prevalence of Alzheimer’s disease (AD), the most prevalent neurodegenerative disorder worldwide. Currently, there are no effective treatment options;

therefore, there is an urgent need for novel, safe, and efficacious strategies to mitigate this disorder. Recent work in a mouse model of Down syndrome, which develops some aspects of AD-like phenotype, has shown that maternal choline supplementation (MCS), during gestation and lactation leads to an improvement in cognitive function in 12-month-old offspring. Here, we examined MCS effects on learning and memory in APP/PS1 mice, a widely used mouse model of AD. Specifically, APP/PS1 breeding pairs were kept on a CTL diet (choline normal diet, with standard choline content of 1.1 g/kg choline chloride) or on a MCS diet (5 g/kg choline chloride) from conception through postnatal day (PND) 21. At PND 21, all offspring were put on the CTL diet for 11 months, after which they were tested in spatial reference memory using the Morris water maze task (MWM). We found that APP/PS1 mice whose mothers were on the MCS diet performed significantly better than APP/PS1 mice whose mothers were on the CTL diet. The cognitive improvement was associated with reduced Aβ plaques and β-secretase activity. Collectively, our results provide preliminary support suggesting that simply modifying the diet of pregnant mothers with additional choline might help reduce the risk of developing AD and/or delay the onset of the disease for offspring in late adulthood; benefits which may be carried over throughout subsequent generations.

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Poster

513. Alzheimer's Disease: Therapeutics

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Program#/Poster#: 513.02/X5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RO1 AG037637

Title: p62 gene transfer improves AD-like pathology by increasing autophagy

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Abstract: The multifunctional protein p62 is associated with neuropathological inclusions in several neurodegenerative disorders, including frontotemporal lobar degeneration, amyotrophic lateral sclerosis, and Alzheimer’s disease (AD). Strong evidence shows that in AD, p62 immunoreactivity is associated with neurofibrillary tangles and is involved in tau degradation. However, it remains to be determined whether p62 also plays a role in regulating amyloid-β aggregation and degradation. Using a gene therapy approach, here we show that increasing brain

p62 expression rescues cognitive deficits in APP/PS1 mice, a widely used animal model of AD. The cognitive improvement was associated with a decrease in amyloid-β levels and plaque load. Using complementary genetic and pharmacologic approaches, we found that the p62-mediated changes in Aβ were due to an increase in autophagy. To this end, we showed that removing the LIR domain of p62, which facilitates p62-mediated selective autophagy, or blocking autophagy with a pharmacological inhibitor, was sufficient to prevent the decrease in Aβ. Overall, these data provide the first direct *in vivo* evidence showing that p62 regulates Aβ turnover.

Disclosures: **A. Caccamo:** None. **E. Ferreira:** None. **C. Branca:** None. **S. Oddo:** None.

Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 AG037637

Title: Dissecting the role of Nrf2 in Alzheimer’s disease

Authors: ***C. BRANCA**, A. CACCAMO, C. A. NEGRICH, N. DAVE, S. ODDO;
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Abstract: Alzheimer's disease (AD) is the most common type of dementia in the elderly. Growing evidence links oxidative stress and free radical damage to the initiation and progression of AD; however the molecular mechanisms underlying the link between oxidative stress and AD pathogenesis remain elusive. Nrf2 is a master transcriptional regulator that controls the expression of several genes involved in antioxidant response. To investigate the involvement of Nrf2 in AD pathogenesis, we used a crossbreeding strategy to remove the Nrf2 gene from the brain of APP/PS1 mice, a widely used model of AD. We found that removing both copies of the Nrf2 gene from the APP/PS1 mice increased behavioral deficits in several tasks, such as Morris water maze, radial arm water maze, and contextual fear conditioning. These deficits correlated with an increase in amyloid beta (Aβ) pathology, without affecting the APP processing. Mechanistically, we found that the increase in Aβ levels was not due to changes in turnover, as we found that autophagy induction and proteasome activity were similar between APP/PS1 with and without Nrf2. In contrast, we found that APP/PS1 mice lacking Nrf2 had higher levels of activated microglia and astrocytes, suggesting that the increase of Aβ might be mediated by an increase in brain inflammation.These results provide a better understanding of the role of Nrf2, and oxidative stress in general, in the progression of AD.

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Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AACP New Investigator Award

Title: Targeting phosphodiesterase 2 for treatment of memory loss by regulating neuroplasticity

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Abstract: Alzheimer's disease (AD) is characterized by progressive cognitive impairment, which is the most common cause of dementia in the aging population. AD is the sixth leading cause of death in the United States and the mortality rate has increased by 70% in during the last ten years. To date, no treatments are available to cure or halt the progression of AD. Synaptic loss, a detectable trait in patients with mild cognitive impairment, is the major neurobiological substrate of cognitive dysfunction. The dysfunction of cAMP and/or cGMP signaling in the progress of AD reduces synaptic firing and rapidly affects cognitive function. In the present study, we investigated the effects of PDE2 inhibitor Bay 60-7550 on memory performance in a mouse model of AD by microinfusion of beta amyloid peptide 1-42 (Abeta-42) into CA1 subregions of the hippocampus. Memory performance was measured by Morris water maze and passive avoidance tests, which are sensitive to evaluate PDE2 inhibitors. The results showed that Bay 60-7550 rescued memory deficits induced by Abeta-42, as evidenced by shorter latencies and more platform crossings in the target quadrant of the probe trails and longer step-down latency to avoid electric footshock tested 24 h after training in Morris water maze and passive avoidance tests, respectively, following treatment with Bay 60-7550. Bay 60-7550 also reversed Abeat-42-induced synaptic and neuronal dysfunction, i.e. increases in the number of dendrites and the total length of neuritis, as well as the spine density in the hippocampus. Immunoblot analysis revealed that Bay 60-7550 also reversed decreased CREB and BDNF expression in the hippocampus induced by Abeta-42. These findings provide evidence for the role of PDE2 in mediating cognitive impairment associated with AD by regulating synaptic and neuronal plasticity, which implicate potential novel therapeutic strategies for treating AD.

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Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: SUVN-D4010, a novel 5-HT_4 receptor partial agonist: preclinical profile demonstrating symptomatic and disease modifying effects in the treatment of alzheimer's disease

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Abstract: SUVN-D4010 is a potent, selective and orally bioavailable 5-HT_4 receptor partial agonist being developed for the treatment of Alzheimer’s disease. The effect of SUVN-D4010 on the cholinergic neurotransmission was studied using brain microdialysis technique. The procognitive effect of SUVN-D4010 was evaluated using object recognition task (ORT). The effect of SUVN-D4010 on the oscillatory activity in the hippocampus of anesthetized rats was evaluated using EEG. The effect of SUVN-D4010 on the toxic beta amyloid and the neuroprotective sAPPα were evaluated in the preclinical species using ELISA kits. SUVN-D4010 increased cortical acetylcholine levels in freely moving rats and the effects were blocked by GR 125478, a selective 5-HT_4 receptor antagonist. Similarly, the procognitive effects in ORT were blocked by GR 125478. This indicates that the observed efficacy is mediated through the target receptor. Co-administration of SUVN-D4010 potentiated donepezil’s effects on extracellular acetylcholine levels. SUVN-D4010 also potentiated the effects of donepezil on stimulation elicited theta oscillatory activity in anesthetized rats. At therapeutically effective doses, significant increase in cortical sAPPα and decrease in toxic beta amyloid protein levels were observed in preclinical species. SUVN-D4010 also demonstrated antidepressant like effects in animal models of depression such as forced swim test. These findings in preclinical animal models suggest that SUVN-D4010 exhibits both symptomatic and disease modifying properties together with antidepressant properties which may be beneficial in the treatment of Alzheimer's disease. Phase-1 studies have been completed under US IND and SUVN-D4010 was found to be safe and well tolerated in healthy adult subjects.

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Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Bezmialem Vakif University Scientific Research Council grant 12.2014/2

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Title: The effects of thymoquinone in the amyloid beta-induced sporadic alzheimer models in rats

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Abstract: Alzheimer’s Disease is the most common neurodegenerative disease in aged population. This disease characterized by synaptic deficiency, neuronal damage and neuron loss in the hippocampus, a brain region specific for memory formation, has no effective treatment. It was known that existing molecules for treatment of Alzheimer’s Disease provide only symptomatic recovery. Nowadays, the investigations of neuroprotective substances preventing Alzheimer’s disease-induced neuronal damage gain importance. One of these candidate molecules is Tymoquinone (TQ), an aromatic hydrocarbon which is found in the *Nigella Sativa*. In literature, the effectiveness of TQ in the treatment of Alzheimer’s Disease was investigated *in vitro* without any *in vivo* studies. Therefore, the aim of the present study was to examine the molecular principles of the neuroprotective effects of TQ in the hippocampus of rats which have

sporadic Alzheimer's disease. In the current study, a micro-osmotic pump which include aggregated amyloid beta was placed to the hippocampus of 6 month-old rats. During 15 days, TQ at a dosage of 20 mg/kg/day were intubated intragastrically as a treatment. After behavioral tests (Morris Water Maze (MWM) and Passive Avoidance Test (PAT)), the rats were decapitated and their brains were removed for histological and molecular analysis. The formation of amyloid plaques was visualized by anti-amyloid beta antibody fluorescently. To confirm the results, a congo red staining were performed to the brain sections. The neuronal loss in the hippocampus was determined by cresyl violet staining. Lastly, the RT-PCR were used to determine the changes in the expression levels of selected microRNAs (mir26b, mir29a, mir29c, mir124) to obtain the molecular effect of TQ in the treatment of Alzheimer pathology. In the TQ-treated Alzheimer rats, there was a functional recovery in the memory performance which was obtained from probe trial of MWM while there was no significant difference in the escape latency in both PAT and MWM learning compared to untreated rat with Alzheimer's disease. According to immunohistochemical analysis, TQ decreased amyloid beta plaques in the hippocampus. Interestingly, TQ increased the expression of mir124 gene whose concentration is altered in the neuronal plasticity in the hippocampus of Alzheimer rats. In conclusion, TQ may be a candidate molecule in the treatment of Alzheimer's disease due to its capacity to recover AD-related neuropathology and to increase the expression of synaptic plasticity molecules.

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Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Significant Aβ42 lowering in PS1-E280A knock-in mouse brain following γ-secretase modulator administration

Authors: *K. M. WOOD, M. SHEEHAN, C. GONZALES, N. I. POZDNYAKOV, M. CAI, S. HASSON, M. PETTERSSON, E. HAJOS-KORCSOK, K. R. BALES;
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Abstract: We developed a mouse model utilizing CRISPR technology to knock-in the familial Alzheimer's Disease (FAD) mutation, Presenilin 1-E280A (PS1-E280A). In humans, the PS1-E280A mutation results in an aggressive, early onset form of AD caused by a shift in Aβ cleavage, leading to an increase in the production and deposition of Aβ42 in brain. We

investigated the impact of the PS1-E280A mutation on mouse brain Aβ production and assessed the effect of γ-secretase modulator (GSM) treatment on the various forms of Aβ. Previously published efficacy data from multiple preclinical models provided evidence for a potentially beneficial effect of GSM treatment in AD by shifting cleavage away from amyloidogenic forms Aβ42 and Aβ40 toward production of carboxy-terminal truncated forms Aβ37 and Aβ38; these shorter fragments are considered less amyloidogenic with reduced neurotoxicity. The PS1-E280A mutation in mouse resulted in modulation of brain Aβ production, with increased Aβ42 and decreased Aβ37 and Aβ38. GSM treatment in PS1-E280A mice had a beneficial effect by correcting the abnormal Aβ cleavage shift caused by the FAD mutation.

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Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH T32 Grant AG000216

Title: Combination therapies with γ-secretase modulator for alzheimer's disease

Authors: *O. PRIKHODKO[1], M. Y. VO[2], P. NGUYEN[3], K. RYNEARSON[3], L. MONTE[3], R. E. TANZI[4], R. RISSMAN[3], S. L. WAGNER[3];
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Abstract: Alzheimer's disease (AD) is the most prevalent form of dementia in the elderly, which affects about 5.4 million individuals in the US. Current AD treatments are limited to temporary and mild alleviation of cognitive and behavioral symptoms in a subset of patients; there is no cure, effective prevention therapy or treatment which alters the course of AD. The pathological features of AD include β-amyloid (Aβ) plaques and neurofibrillary tangles in cerebral cortex and hippocampus. Aβ peptides are the product of the amyloid precursor protein (APP) cleavage by BACE1 (β-site APP cleaving enzyme 1) to produce the membrane-bound C-terminal fragment C99, which is further processed by γ-secretase to generate Aβ fragments. Aβ40 is the most abundant secreted Aβ peptide; however, the more fibrillogenic Aβ42 is the primary constituent of Aβ plaques. Our group developed and tested a series of small molecule γ-secretase modulators (GSMs), with BPN15606 emerging as the leading candidate for clinical development. BPN15606 demonstrates excellent in vivo PK/PD properties, highly significant dose-dependent biochemical efficacy and dose proportional exposures. At higher doses, this compound can almost completely eliminate Aβ42 levels in both brain and in CSF. In the current study, we evaluated the benefit of two distinct combinational therapies. The first therapy uses BPN15606 and a BACE1 inhibitor, LY2886721, in multiple in vitro assays and in an animal study currently in progress. The second therapy combines BPN15606 and a CRFR1 inhibitor (cotricotropin-releasing factor receptor 1, which modulates cellular activity in many AD-relevant brain areas, and has been demonstrated to impact both tau phosphorylation and Aβ pathways) in an in vivo study. Collectively, the presented data demonstrates the improved efficacy of combination therapies over single-therapy treatments for AD.

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Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Effects of uric acid on the cellular changes caused by amyloid beta

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Abstract: Alzheimer's disease (AD) is the most common cause of degenerative dementia, which is characterized by the accumulation of beta-amyloid plaques and tau tangles. Uric acid (UA), the final product of purine metabolism, is a well-known natural antioxidant that reduces oxidative stress and protects cells against free radicals. Recent studies raised a possibility that UA could have beneficial effects on the cognitive decline in patients with AD pathology. However, it is currently unknown whether UA protects neurons from the toxic effects of AD pathology. In this study, we investigated the protective effects of UA using in vitro models of AD. UA increased the survival of neurons from cell death induced by amyloid beta. UA also reversed the elevation of ROS induced by amyloid beta. Furthermore, UA increased the activity of SOD, and maintained the potential of mitochondrial membrane. These results suggest that UA protects cells against cell death induced by amyloid beta.

Disclosures: B. Ye: None. **D. Kim:** None. **P. Lee:** None.

Poster

513. Alzheimer's Disease: Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 513.10/X13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FAPESP 2014/14661-1

Title: Anandamide prevents memory impairment induced by streptozotocin in a model of sporadic Alzheimer's disease in rats.

Authors: D. MOREIRA-SILVA[1], D. C. CARRETTIERO[2], A. S. A. OLIVEIRA[3], S. RODRIGUES[1], A. P. MOTZKO-SOARES[1], *T. L. FERREIRA[4,1];
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Abstract: The endocannabinoid system has emerged as important modulator of memory processing and changes in the endocannabinoid system have been correlated to cognitive deficits in Alzheimer's disease (AD). However, it remains unknown how the exogenous administration of anandamide (ANA) could modulate the development of memory impairments in AD subjects. This study aimed to investigate the preventive effects of ANA in a sporadic AD model induced

by streptozotocin (STZ; which promotes brain resistance to insulin) on recognition and emotional memories tasks. Adult male Wistar rats (300-400g) received intracerebroventricular injections of ANA (100 ng) and/or STZ (2.0 mg/kg) or vehicle (groups: Veh/Veh, Veh/STZ, ANA/Veh and ANA/STZ). After 30 days, animals were submitted to object recognition task and two days later, they were trained in fear conditioning paradigm and tested 24 hours (contextual) and 48 hours late (tone). No differences were observed in locomotion or exploration in STZ and/or ANA treated animals. STZ impaired short-term recognition memory and ANA prevented STZ-induced deficit because animals treated with STZ demonstrated a lower recognition index compared to all groups. ANA *per se* did not affect object recognition memory. Emotional memory evaluated by contextual and tone fear conditioning was not affected by STZ and/or ANA. Our data showed that, after 30 days, STZ disturbs recognition but not fear memories, suggesting that hippocampus but not amygdala memory systems are more sensitive to cognitive deficits showed by AD model used in this study. Importantly, ANA prevented this impairment on recognition memory induced by STZ. Molecular analyses (tau, p-tau and co-chaperones) are in progress to understand underlying biological processes related to our results.

Disclosures: D. Moreira-Silva: None. **D.C. Carrettiero:** None. **A.S.A. Oliveira:** None. **S. Rodrigues:** None. **A.P. Motzko-Soares:** None. **T.L. Ferreira:** None.

Poster

513. Alzheimer's Disease: Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 513.11/X14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ohio University SEA Grant

Ohio University OURC Grant

Title: Zinc and pyrithione exposure prevents 2,2’-dithiodipyridine-induced varicosity formation in rat primary cortical neurons

Authors: *C. QIAN, R. A. COLVIN;
Biol. Sci., Ohio Univ., Athens, OH

Abstract: Varicosities along neurites are early-stage traits of degenerating neurons found in AD and ALS. One common trigger of these swellings is microtubule disruption, leading to microtubule-associated protein dissociation and disruption of axonal transport. Zinc has been reported to stabilize microtubules and regulate axonal transport, but mechanisms remain

unknown. Visualizing tau by immunofluorescence (IF) showed tau colocalized with varicosities formed in rat primary cortical cells after 15min treatment with 60μM 2,2'-dithiodipyridine (DTDP), which has been reported to undergo thiol-disulfide exchange reaction with cysteines (thiol) on either tau or tubulin. Imaging analysis showed that the number of tau-positive varicosities increased by 841 ± 21%. DTDP increased tau fluorescence by 159 ± 22% in cell bodies and 166 ± 8% in neurites (mean ± SEM, n = 3). Western-blots after DTDP treatment showed high molecular weight βME-sensitive bands of presumptive tau aggregates. Furthermore, IF showed that tubulin and MAP2 were co-localizing with tau in the varicosities. DTDP treatment (15min) did not cause accumulation of transporting cargoes mitochondria or postsynaptic markers PSD-95 in the varicosities - the overall distribution could not be distinguished from control. Hydrogen peroxide (200μM) did not lead to varicosity formation after 15min treatment, suggesting that DTDP's general oxidative capacity is not the causation, and varicosity formation is not simply a morphological sign caused by common pathways leading to cell death. In addition, the role of DTDP-induced intracellular free zinc and calcium increase was studied - either zinc-specific chelator TPEN (100μM) or zinc/calcium chelator BAPTA-AM (10μM) failed to prevent varicosity formation. Surprisingly, exposing cells to zinc entry mediated by zinc ionophore pyrithione (10μM zinc/5μM pyrithione) prevented DTDP-induced varicosity formation, increases in tau immunofluorescence, but did not alter tau aggregation. Adding zinc lower than 10μM or in the absence of pyrithione was not protective. In conclusion, DTDP-induced tau dissociation (increased tau immunofluorescence) and aggregation are likely triggered by tau disulfide-crosslinking directly, or by microtubule disruption indirectly. Intriguingly, intracellular free zinc released from physiological binding sites attacked by DTDP is not a mediator of the observed effects, but adding zinc prevents varicosity formation implying zinc somehow protects critical cysteines on tubulin. However, adding exogenous zinc did not block tau aggregation, suggesting zinc may not interfere with the DTDP-induced disulfide-crosslinking of tau.

Disclosures: C. Qian: None. **R.A. Colvin:** None.

Poster

513. Alzheimer's Disease: Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 513.12/X15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Mary S. Easton Center for Alzheimer's Disease Recruitment Fund

Title: sAPPα is an allosteric inhibitor of BACE1 and potential therapy for Alzheimer's disease.

Authors: *P. SPILMAN[1,2], J. CAMPAGNA[1], C. PETERS-LIBEU[2], M. P. ALAM[1], M. ARBING[3], S. CHAN[3], M. COLLAZO[3], D. CASCIO[3], D. BAI[1], V. JOHN[1];
[1]Neurol., Mary S Easton Ctr. for Alzheimer's Dis. Res. at UCLA, Los Angeles, CA; [2]Bredesen Lab., Buck Inst. for Res. on Aging, Novato, CA; [3]Inst. for Genomics and Proteomics, Univ. of California, Los Angeles, CA

Abstract: Amyloid precursor protein (APP) may be processed via an amyloidogenic pathway wherein it is cleaved sequentially by β-secretase BACE1 (BACE) and γ-secretase to form amyloid-β (Aβ), the major component of the amyloid plaques that characterize Alzheimer's disease (AD). Alternatively, APP may be cleaved by α-secretase to generate sAPPα and αCTF. As sAPPα supports synaptic and cell survival, we focused our drug discovery efforts on identification sAPPα enhancers as new potential therapeutics for AD. We observed sAPPα increases in brain induced by F03 (Spilman et al. 2013) and analogs after 28-day oral delivery at 4 mkd to J20 AD model mice correlated well with decreases in sAPPβ, suggesting sAPPα may affect BACE cleavage of APP. In 2012, Obregon et al. revealed sAPPα did indeed interact with and inhibit BACE; and we showed by small-angle X-ray scattering (SAXS) and tryptophan fluorescence studies that this inhibition was conformation-dependent and allosteric in nature, that is, sAPPα interacts with an exosite remote from the active-site of BACE. The latter was revealed by sAPPα's inhibition of BACE cleavage of a longer MBP-APPC125, but not a shorter P5-P5', substrate (Peters-Libeu et al. 2015). To further elucidate the sAPPα:BACE interaction, we have undertaken co-crystallization studies using recombinant sAPPα and rBACE (Sussman et al. 2013). In addition, as our ultimate goal is to develop sAPPα as a potential therapeutic for AD and as a protein fragment it is not suitable for conventional delivery, we have encapsulated rsAPPα in small, uniform, BBB-targeted elastic liposomes using a microfluidic reactor to allow delivery to brain after peripheral injection. sAPPα's ability to inhibit BACE cleavage of APP - the first and rate-limiting step in Aβ production - by a physiologically relevant mechanism makes it a potential biologic therapeutic for AD. In addition, the allosteric mechanism provides the potential for both greater enzyme- and substrate specificity, lowering the risk of off-target effects. Elucidation of the nature of the interaction would provide information to support therapeutic development. Furthermore, early results indicate liposomal encapsulation may provide a method for successful peripheral delivery of sAPPα.

Disclosures: P. Spilman: None. **J. Campagna:** None. **C. Peters-Libeu:** None. **M.P. Alam:** None. **M. Arbing:** None. **S. Chan:** None. **M. Collazo:** None. **D. Cascio:** None. **D. Bai:** None. **V. John:** None.

Poster

513. Alzheimer's Disease: Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 513.13/X16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: LIPI-NAT-VIEP-BUAP, 2015-2016

Title: The C-terminal fragment of tetanus toxin protects against Amyloid-β (25-35) peptide through the Akt/GSK-3β pathway

Authors: *A. PATRICIO[1], I. MARTÍNEZ[1], O. REYES CASTRO[1], L. MENDIETA[1], V. ALEMÁN ALEMÁN[2], J. AGUILERA[3], I. D. LIMÓN[1];
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Abstract: The C-terminal domain of tetanus toxin (Hc-TeTx) is a nontoxic fragment of the tetanus toxin that has a protective action against excitotoxicity *in vitro* and *in vivo*. The efficacy of Hc-TeTx fragment it has been demonstrated in several animal models of neurodegeneration. For this reason, the Hc-TeTx fragment it has been proposed as a molecule with neuroprotective and neurorestaurative potential. The $A\beta_{25-35}$ fraction mimics the toxic effects of the full complete peptide Aβ. This fraction modify the cholinergic system in magnocelular nucleus and results in cognitive impairments. The aim of this study was evaluate the neuroprotective effect of Hc-TeTx fragment against toxicity of $A\beta_{25-35}$ fraction into NBM in rats. Male Wistar rats were administered bilaterally with 2μL of $A\beta_{25-35}$ peptide at a concentration of [1μg /μL] (n = 12) into NBM by stereotaxic surgery estereotáxica (AP: -0.6, L: ±2.7, P: -6.5) and Hc-TeTx [2μM]+$A\beta_{25-35}$ [1μg/μL] group. Animals were tested for spatial learning and memory in the eight arm radial maze. The brains were obtained to assess expression and phosphorylation such as Akt (Ser 473), GSK-3β (Tyr 216), Trk-A receptor (Tyr 674/675) and vesicular acetylcholine trasporter (VAChT) by western-blot in NBM, frontal and temporal cortex (CxF, CxT). Finally a group of animals was designed to evaluate the activity of acetylcholinesterase (AChE) by the Ellman assay in Cx-F and CxT. We found that the administration of Hc-TeTx+$A\beta_{25-35}$ prevents a decrease in phosphorylation of Trk-A receptor and Akt protein, on the other hand decreases the phosphorylation of GSK-3β and maintains expression of VAChT compared with $A\beta_{25-35}$. All these improvements could be reflected on spatial learning and memory performances. In summary, our findings suggest that Hc-TeTx fragment promotes Trk-A signaling and improves the spatial learning and memory process in rats with $A\beta_{25-35}$ peptide.

Disclosures: **A. Patricio:** None. **I. Martínez:** None. **O. Reyes Castro:** None. **L. Mendieta:** None. **V. Alemán Alemán:** None. **J. Aguilera:** None. **I.D. Limón:** None.

Poster

513. Alzheimer's Disease: Therapeutics

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 513.14/X17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Newton-Bhabha 2015 grant

Title: The effect of phloretin, a phytoestrogen, on amyloid beta 1-42 induced toxicity in primary cortical neurons and *In vivo* models

Authors: *P. J. GHUMATKAR, JR[1], V. PESHATTIWAR, Jr[1], D. WHITFIELD[2], S. SATHAYE[1], P. FRANCIS[2];
[1]Inst. of Chem. Technol., Mumbai, India; [2]King's Col. London, London, United Kingdom

Abstract: The aggregation of amyloid beta (Aβ1-42) that results in formation of extracellular senile plaques is the major pathological hallmark of Alzheimer's disease (AD). Aβ1-42 has been shown to produce deleterious effect on hippocampal adult neurogenesis, synaptic proteins and cognition. Phloretin, a phytoestrogen found in apple leaves has previously been shown to enhance spatial memory and to demonstrate strong antioxidant, anti-inflammatory and neurotrophic actions in a scopolamine induced amnesia mouse model. The objective of this study was to evaluate the effect of phloretin on Aβ1-42 induced pathological alterations *in vitro* as well as *in vivo*. In this study, we examined the ability of phloretin to protect primary cortical neuronal cells exposed to oligomeric Aβ1-42 as well as investigated a potential modulatory role on adult neurogenesis in Aβ1-42 injected Wistar rats. In the in vitro study, primary rat cortical neurons were treated with phloretin 10μM and then further exposed to Aβ1-42 oligomers 3μM concentration. For the in vivo study, male Wistar rats were pre-treated with phloretin 5mg/kg, followed by intra-hippocampal injections of 2 μL of aggregated Aβ1-42 at 200 μM/L concentration. Following sacrifice, perfused brains were removed and subjected to immunohistochemical analysis for BrdU, Ki67 and doublecortin (DCX). Exposure of primary neuronal cultures (n=3) for 24 hours to Aβ1-42 significantly ($P \leq 0.01$) decreased the cell viability by 19% as compared to the vehicle control cells. However, 3 days pretreatment with phloretin significantly ($P \leq 0.001$) protected the neuronal cells from the Aβ1-42 toxicity. The preliminary results of the in vivo study demonstrated that 21 days phloretin treatment elevated the number of BrdU, Ki67 and DCX positive neurons in the dentate gyrus as compared to sham control rats by 70%. Aβ1-42 injections decreased cells stained for BrdU and Ki67 as well as DCX in the sub

granular zone (sgz) of the hippocampus by a minimum of 35%. Pretreatment of Aβ1-42 injected rats with phloretin increased the number of DCX positive cells by 42% in the sgz of when compared to Aβ1-42 rat brains. The above experimental findings point to a neuroprotective role of phloretin in Aβ1-42 exposed primary cortical neurons and an ability to enhance adult hippocampal neurogenesis in normal as well as Aβ1-42 injected rats. While further work is required these encouraging preliminary findings highlight the potential of phloretin as a dietary supplement targeting key therapeutic mechanisms in AD.

Disclosures: P.J. Ghumatkar: None. **V. Peshattiwar:** None. **D. Whitfield:** None. **S. Sathaye:** None. **P. Francis:** None.

Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC CGS-D to ALM

NSERC Discovery Grant to NJM

Title: Neurosteroid metabolites of testosterone and progesterone are protective against amyloid β-induced neurotoxicity

Authors: *A. L. MENDELL, C. E. CREIGHTON, N. J. MACLUSKY;
Biomed. Sci., Univ. of Guelph, Guelph, ON, Canada

Abstract: Androgens, estrogens, and progestogens have all been reported to have neuroprotective properties. It has been suggested that the protective effects of androgens and progestogens may involve conversion to neurosteroid metabolites in the brain. 5α-androstane-3α,17β-diol (3α-diol) and 3α-hydroxy-5α-pregnan-20-one (allpregnanolone; ALLO), metabolites of testosterone and progesterone, respectively, have been shown to allosterically potentiate the activity of GABA at the $GABA_A$ receptor ($GABA_AR$). Intriguingly, elevated free levels of these hormones have been correlated with a reduction in the risk for Alzheimer's disease. Dysregulation and prolonged phosphorylation of extracellular signal-regulated kinase (ERK) is an indication of oxidative stress, and has been implicated in amyloid-induced neurotoxicity. In this study, we sought to determine whether 3α-diol and ALLO could protect against prolonged ERK phosphorylation induced by hydrogen peroxide (H_2O_2) and amyloid β peptide 1-42 (Aβ42) in SH-SY5Y human neuroblastoma cells and primary cortical neurons cultured individually from

male and female mice. Both 3α-diol and ALLO prevented prolonged ERK activation induced by 24 hour H_2O_2 treatment and 48 hour Aβ42 treatment in SH-SY5Y cells and primary cortical neuron cultures from male or female mice. However, the concentration required for these effects differed drastically - a final concentration of 100nM was necessary to achieve neuroprotection with ALLO, while 3α-diol was able to produce the same apparent protection at a final concentration of only 10nM, below the threshold for its potentiation of $GABA_A$ signaling. Additionally, antagonism of the $GABA_AR$ prevented the protective effect of ALLO, but not 3α-diol. These results suggest that neurosteroids may play a role in the prevention of amyloid-induced neurotoxicity, and may therefore contribute to the neuroprotective effects of androgens and progestogens. The mechanisms and concentration requirements for ALLO- and 3α-diol-mediated protection appear, however, to be different. The potency of 3α-diol suggests that it may contribute to the apparent reduction in risk of Alzheimer's disease in men with high circulating free testosterone levels (Hogervorst *et al* Exp Gerontol. 2004;39:1633).

Disclosures: A.L. Mendell: None. **C.E. Creighton:** None. **N.J. MacLusky:** None.

Poster

513. Alzheimer's Disease: Therapeutics

Location: Halls B-H

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Program#/Poster#: 513.16/Y1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's society of canada

alzheimer's society of manitoba

canadian diabetes association

Title: Secreted APP alpha overexpressing neural stem cells as a therapeutic for dementia-linked insulin signaling dysfunction

Authors: *B. AULSTON, G. GLAZNER, G. ODERO;
St. Boniface Res. Ctr., Winnipeg, MB, Canada

Abstract: Aberrant insulin signaling is present in the Alzheimer's brain and may underlie disease-associated cognitive dysfunction. Moreover, the administration of exogenous insulin has shown promise as a treatment for Alzheimer's disease (AD) suggesting that activation of brain insulin signaling is a therapeutically viable strategy. Despite mounting evidence that the neurotrophic factor secreted amyloid precursor protein alpha (sAPPα) may activate insulin signaling pathways and reverse diabetes and AD-associated pathology, the therapeutic potential

of sAPPα as a treatment for dementia-linked insulin signaling dysfunction has yet to be explored. Furthermore, there is currently no clinically viable sAPPα delivery system suitable for human patients. Therefore, we tested the effects of sAPPα overexpressing neural stem cells (NSCs) on cognitive dysfunction and disease-induced pathology in mouse models of AD and diabetes. 6 month old, non-transgenic SAMP8 AD mice were made diabetic by i.p. injections of STZ and wild-type or sAPPa-overexpressing NSCs implanted into the hippocampus 2 months later. 8 weeks after NSC implantation, cognitive performance was evaluated in these mice via Morris Water Maze. Mice were then sacrificed and hippocampi were analyzed for activation of insulin signaling, presence of amyloid beta and phosphorylation of tau. Some hippocampi were reserved for analysis of mitochondrial function, LTP and characteristics of NSC transplants. In total, the work presented here supports the development of sAPPα-overexpressing NSCs as a therapeutic for the treatment of dementia-linked insulin signaling dysfunction.

Disclosures: **B. Aulston:** None. **G. Glazner:** None. **G. Odero:** None.

Poster

513. Alzheimer's Disease: Therapeutics

Location: Halls B-H

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Program#/Poster#: 513.17/Y2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Annexin A1 can restores Aβ1-42 induced blood brain barrier disruption

Authors: *J. PARK, S. BAIK, S.-H. HAN, H. CHO, H. CHOI, H. KIM, H. CHOI, W. LEE, D. KIM, I. MOOK-JUNG;
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Abstract: The blood brain barrier (BBB) is composed of brain capillary endothelial cells and has an important role in maintaining homeostasis of the brain separating the blood from the parenchyma of the central nervous system (CNS). It is widely known that disruption of the BBB occurs in various neurodegenerative diseases, including Alzheimer’s disease (AD). ANXA1 (ANXA1), an anti- inflammatory messenger, is expressed in brain endothelial cells and there were some reports that it can regulates BBB integrity. However, its role and mechanisms for protecting BBB in AD have not been identified. We found that β-Amyloid 1-42 (Aβ42)-induced BBB disruption was rescued by human recombinant ANXA1 (hrANXA1) in the murine brain endothelial cell line bEnd.3 and the capillaries of 5XFAD mice. In addition, we verified that the RhoA signaling pathway was activated in the Aβ42 treated bEnd.3 cells and the capillary of 5XFAD mice. Therefore, we suggest that ANXA1 can restore Aβ42-induced BBB disruption through inhibition of RhoA-ROCK signaling pathway and propose ANXA1 as a therapeutic

reagent, protecting against the breakdown of BBB in AD. **Keywords:** Annexin A1; blood-brain barrier; Alzheimer's disease; RhoA-GTP

Disclosures: **J. Park:** None. **S. Baik:** None. **S. Han:** None. **H. Cho:** None. **H. Choi:** None. **H. Kim:** None. **H. Choi:** None. **W. Lee:** None. **D. Kim:** None. **I. Mook-Jung:** None.

Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG048935-01A1

NIA Grant R21AG043813-01A1

Title: Intensive brain training intervention fails to reduce amyloid pathologies or cognitive deficits in transgenic mouse models of Alzheimer's disease

Authors: *M. ANDERSON[1,2], F. XU[1], M. OU-YANG[1], J. DAVIS[1], W. VANNOSTRAND[1], J. ROBINSON[1];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is the leading cause of dementia in the elderly. Amyloid β protein (Aβ) depositions in both the brain parenchyma and the cerebral vasculature are recognized as important pathological components that contribute to the cognitive impairments found in individuals with AD. Because pharmacological options have been minimally effective in treating cognitive impairment to date, interest in the development of preventative lifestyle intervention strategies has increased in the field. One controversial strategy, cognitive-specific stimulation, has been studied previously in human participants and has been widely commercialized in the form of 'brain-training games.' In the present study, we developed a highly controlled, isolated cognitive training intervention program for mice. Two transgenic mouse lines, one that develops Aβ deposition largely in brain parenchyma, and another in the cerebral microvasculature, progressed through a series of domain-specific tasks for an average of 4 months. Following the intervention, spatial memory and exploratory behavior were assessed in the Barnes maze and the DigiScan. Brain tissue was harvested for quantitative ELISA and immunostaining for Aβ species. Despite the high intensity and duration of the intervention, we found little evidence of positive benefits for AD amyloid pathologies and post-training cognitive testing in these two models. Taken together, these results

support the current evidence in human studies that cognitive-specific stimulation does not lead to a measureable reduction in AD pathology or an improvement in general brain health.

Disclosures: **M. Anderson:** None. **F. Xu:** None. **M. Ou-Yang:** None. **J. Davis:** None. **W. VanNostrand:** None. **J. Robinson:** None.

Poster

513. Alzheimer's Disease: Therapeutics

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Program#/Poster#: 513.19/Y4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Design of inhibitor against amyloid toxicity

Authors: ***C. LIU**, D. LI, C. WANG;
Chinese Acad. of Sci., Shanghai City, China

Abstract: Alzheimer disease (AD) is one of the major forms of neurodegenerative disorders. Currently, over 50 million patients are suffering from AD worldwide. The abnormal aggregation of amyloid-beta (Aβ) into plaques in brain is the hallmark of AD[1,2]. The most widely used strategy for drug development against AD is to prevent the formation of pathogenic Aβaggregates[3,4]. In this study, we performed two different strategies of structure-based inhibitor design against Aβtoxicity. One is to develop peptide-based beta-sheet mimics to target the nucleus of Aβaggregates. By blocking the nucleation process, beta-sheet mimics efficiently inhibited Aβaggregation and reduced its cytotoxicity in mammalian cells. The second strategy is to design small molecules capable of stabilizing Aβfibrils. From structure-based rational design, several compounds were identified as tight fibril binders. The compounds were able to detoxify Aβby accelerating the formation of non-toxic amyloid fibril. Further in vivo studies would be necessary to evaluate the potential of these inhibitors as drug candidates. 1. Chiti, F. & Dobson, C. M (2006) Protein misfolding, functional amyloid, and human disease. Annu. Rev. Biochem. **75**, 333-366. 2. Eisenberg D, Jucker M (2012) The amyloid state of proteins in human diseases. Cell **148**(6):1188-1203. 3. Aguzzi, A. & O'Connor, T (2010) Protein aggregation diseases: pathogenicity and therapeutic perspectives. Nature Rev. Drug. Discov. **9**, 237-248. 4. Härd T, Lendel C (2012) Inhibition of amyloid formation. J Mol Biol **421**(4-5):441-465.

Disclosures: **C. Liu:** None. **D. Li:** None. **C. Wang:** None.

Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG18440

Title: Selective targeting of three repeat tau for the treatment of neurodegenerative disorders

Authors: *B. J. SPENCER[1], E. ROCKENSTEIN[2], A. ADAME[2], P. DESPLATS[3], E. MASLIAH[3];
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Abstract: Neurodegenerative disorders with Tau accumulation are a common cause of dementia in the aging population. Alternative splicing can generate two different major forms of Tau containing either 3 or 4 32 amino acid repeats. These 3R or 4R Tau species are differentially expressed in neurodegenerative diseases with Corticobasal degeneration (CBD) and Progressive supranuclear palsy (PSP) primarily expressing the 4R Tau isoform while Pick's Disease (PiD) primarily expresses the 3R Tau isoform. Immunotherapy may prove to be effective at reducing total Tau protein; however, studies with Tau knock out mice and have shown that this strategy might have deleterious effects. Furthermore, down-regulation of Tau in oligodendrocytes by siRNA results in reduced expression of MBP protein leading to reduced myelination. To date, there has been little or no effort to specifically target the 3R Tau protein. We developed a single chain antibody (scFV) specifically recognizing 3R Tau and then further modified this with proven brain transport peptide to facilitate trafficking into the brain. Non-tg and 3R Tau-tg mice receive IP injections of a lentivirus expressing the 3R Tau scFV, LV-3RT-apoB, or LV-control and after 4 weeks were evaluated behaviorally, biochemically and neuropathologically. 3R Tau-tg mice treated with the brain penetrating LV-3RT-apoB displayed improvements in behavior comparable to the non-tg mice in contrast to mice treated with control vector or the non-brain-penetrating scFV expressing vector. By western blot with an antibody against the scFV, mice treated with LV-control or LV-3RT without the brain penetrating apoB tag showed low or no immunoreactivity, in contrast mice treated with LV-3RT-apoB displayed higher levels of immunoreactivity in brain homogenates. Next, brain sections were immunolabeled with antibodies against the scFV. Likewise, mice treated with LV-control or LV-3RT showed low or no immunoreactivity, in contrast mice treated with LV-3RT-apoB displayed immunoreactivity in neuronal cells in the neocortex and hippocampus. To evaluate the beneficial effects of the 3RT-apoB, brain sections were immunostained with antibodies against 3R Tau. The highest levels of 3R Tau were detected in tg mice treated with the LV-control or LV-3RT. In contrast, tg mice

treated with LV-3RT-apoB showed decreased levels of 3R Tau accumulation in neuronal cells in the neocortex and hippocampus. Thus, we have developed the first 3R Tau specific antibody for the treatment of PiD that can reduce Tau accumulation, prevent neuronal degeneration and reverse behavioral deficits in a mouse model of PiD.

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Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Cullen Trust

The Mitchell Center for Neurodegenerative Disease

The Sealy Center for Vaccine Development

Title: Small molecules targeting and preventing tau oligomer formation and toxicity

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Abstract: Neurodegenerative disorders are the leading cause of death and disability in the elderly population. The number of people living with neurodegenerative diseases continues to rise due to increased life expectancy, therefore, finding effective prevention and treatment strategies is becoming increasingly important. The aggregation and accumulation of the microtubule-associated protein, tau, are pathological hallmarks of many neurodegenerative diseases known as tauopathies such as Alzheimer’s disease, Parkinson’s disease and many others. For a long time, neurofibrillary tangles (NFTs)—aggregates of hyperphosphorylated tau—were assumed to be the cause of neuronal toxicity since they correlate with cognitive decline and neuronal loss. Recent studies by our lab and others demonstrate that tau oligomers are the true toxic and propagating species in these diseases. Tau oligomeric structures, aggregates of an intermediate size between monomers and NFTs, are a heterogeneous group of biophysically and conformationally distinct tau multimers that can be present in numerous

conformations termed tau oligomeric strains. Due to the dynamic nature of these strains, studies focusing on the mechanisms underlying their formation and characteristics are challenging. Importantly, different strains could potentially explain how the aggregation of the same protein causes different diseases, progression rates and phenotypes, even between individuals within the same disorder. Thus, depleting the disease-relevant structures by using small molecules could be a powerful therapeutic strategy that targets toxicity regardless of the diverse factors involved in the formation of tau oligomeric strains. We hypothesize that small molecules targeting and specifically binding to tau oligomeric strains can neutralize their formation and toxicity, thus preventing the spread of pathology. We used biochemical and biophysical methods to characterize tau oligomeric strains and their reactivity with tau oligomer-specific polyclonal and monoclonal antibodies, T22 and TOMA respectively, in the presence and absence of our first small molecule, CA. Interestingly, CA binds and significantly decreases levels of both 3R and 4R oligomeric tau. We identified the first leading compound that is capable of modifying tau oligomeric structures and we are currently developing new compounds based on CA structure. Moreover, we are screening additional compounds for their ability to target and modulate tau oligomeric strains toxicity and/or formation in order to develop disease-specific and personalized therapeutics and imaging reagents.

Disclosures: **F. Lo Cascio:** None. **J.E. Gerson:** None. **U. Sengupta:** None. **G. Taglialatela:** None. **R. Kayed:** None.

Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA Merit BX001257

R01 AG021975

Title: Effects of tau peptide aggregation inhibitor in human tau transgenic models

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Veterans Greater Los Angeles Healthcare Syst., Los Angeles, CA; [5]Neurol., Baylor Col. Med., Houston, TX; [6]Dept. Chem. and Biochem., UCLA, Los Angeles, CA

Abstract: Neurodegeneration and cognitive decline in Alzheimer disease is paralleled by the spread of neurofibrillary tangles or tauopathy that may be initiated but not halted by Abeta directed therapies. Tangles comprised of microtubule-associated protein tau are a tauopathy hallmark, but preclinical studies have implicated smaller, more soluble toxic tau aggregate species including ptau and tau oligomers in the spread of tauopathy and cognitive deficits. Because most aggregation inhibitors have off-target effects that have historically prevented successful drug development, a selective tau aggregation inhibitor, D-TLKIVW (D-peptide), was designed that very specifically targets the aggregate-forming steric zipper region of tau (Sievers et al. 2011). To test *in vivo* efficacy, D-peptide or its TAT-analog (TAT-D) were fed to Drosophila expressing human non-mutant human tau (htau Tg+ Dr) with a degenerative rough eye phenotype. D-peptide reduced tau dimer and protected from rough eye while TAT-D was more protective. Next D-peptide or its scrambled form (d-TAKIVW) as a control were intra-cerebroventricularly infused for two weeks into aged htau Tg+ mice on a mouse tau knockout background (17-20 month, n=14). The ipsilateral hemisphere containing the infusion cannula was used for immunocytochemistry, while the contralateral hippocampus, frontal subcortex and posterior subcortex were selected for biochemical measurements. D-peptide infusion reduced soluble tau dimer, insoluble tau aggregates in subcortex, and phosphorylated tau (pTau, CP13) by ICC in pyramidal neurons of hippocampal CA3. D-peptide increased subcortical tyrosine hydroxylase (TH) and parameters involved in or impacted by anterograde fast axon transport (FAT), a putative tau aggregate target. These included the motor protein kinesin and the mitochondrial marker porin evaluated by Western as well as the perikayal accumulation of amyloid protein precursor (APP) in CA3 and globus pallidus evaluated by ICC. D-peptide also resulted in an increase of post-synaptic protein PSD95 in frontal subcortical fractions. These results suggest that acute D-peptide treatment can limit candidate toxic tau aggregate species *in vivo* to protect neurons in mice and flies with no reduction in total soluble tau. While the present proof of principle study used invasive intraventricular infusion to deliver the drug to mice, if brain penetrant D-peptide or related drugs can be further developed, this structure based drug design may be a viable approach to treating tauopathy, including AD.

Disclosures: S.A. Frautschy: A. Employment/Salary (full or part-time): University of California, Los Angeles, Veterans Greater Los Angeles Healthcare System. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; VA Merit BX001257, R01AG20175. **S. Hu:** A. Employment/Salary (full or part-time): University of California, Los Angeles. **M.R. Jones:** A. Employment/Salary (full or part-time): Veterans Greater Los Angeles HC. **F. Yang:** None. **P. Chen:** None. **G.R. Jackson:** A. Employment/Salary (full or part-time): Baylor College Medicine. **J. Cheng:** A. Employment/Salary (full or part-time): University of California, Los Angeles. **D. Eisenberg:** A. Employment/Salary (full or part-time): UCLA. **G.M. Cole:** A. Employment/Salary (full or part-time): University of California, Los Angeles and Veterans Administration.

Poster

513. Alzheimer's Disease: Therapeutics

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Phenotypic screening for novel inhibitors of Tau aggregation in human iPSC-derived neurons

Authors: *B. VOLETI, M. USENOVIC, L. WARREN, A. WALJI, S. SUR, P. YI, J. LI, H. ZHOU, J. SCHACHTER, B. LUO, L. LUBBERS, S. P. BATTEUR;
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Abstract: Tauopathies are neurodegenerative diseases characterized by the presence of aggregates of abnormally hyperphosphorylated tau. Due to the intrinsic differences in clearance mechanisms between neuronal and non-neuronal cells, we used human iPSC-derived neurons to develop a biologically relevant cellular model that recapitulates the disease. AAV8-mediated overexpression of both wild type (hu tau WT) and an aggregation-prone construct of tau (hu Tau-mod) in iPSC-neurons resulted in disease-relevant phenotypes such as tau phosphorylation, misfolding, aggregation and subsequently neuronal death. Phenotypic screening of a selected library of annotated compounds in the *in vitro* model of human iPSC-derived neurons resulted in ~7% hit rate with targets spanning across different functional classes such as autophagy, kinase inhibition, inflammation etc. Dose-response evaluation of the hits and confirmatory screening assays are ongoing to identify novel targets/pathways and support target identification efforts. In addition, we have established the translation of this model in vivo and overexpression of both WT and aggregation-prone construct of tau in rat hippocampus showed increased tau phosphorylation, misfolding and neurodegeneration at 6 weeks post AAV infusion. Behavioral, biochemical and immunohistochemical analyses are ongoing to further characterize the *in vivo* model of tauopathy. Our goal is to uncover key targets involved in the clearance of tau aggregates that could become potential strategies to cure neurodegenerative disorders associated with tau.

Disclosures: **B. Voleti:** A. Employment/Salary (full or part-time): Merck and Co. **M. usenovic:** A. Employment/Salary (full or part-time): Merck and Co. **L. Warren:** A. Employment/Salary (full or part-time): Merck and Co. **A. Walji:** A. Employment/Salary (full or part-time): Merck and Co. **S. Sur:** A. Employment/Salary (full or part-time): Merck and Co. **P. Yi:** A. Employment/Salary (full or part-time): Merck and Co. **J. Li:** A. Employment/Salary (full or part-time): Merck and Co. **H. Zhou:** A. Employment/Salary (full or part-time): Merck and Co. **J. Schachter:** A. Employment/Salary (full or part-time): Merck and Co. **B. Luo:** A. Employment/Salary (full or part-time): Merck and Co. **L. Lubbers:** A. Employment/Salary (full

or part-time): Merck and Co. **S.P. Batteur:** A. Employment/Salary (full or part-time): Merck and Co.

Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer Society of Canada

Canadian Institutes of health research MOP-126001

Title: Chronic Angiotensin IV administration rescues cerebrovascular deficits and memory retention in a mouse model of Alzheimer's disease

Authors: *J. ROYEA, M. LACALLE-AURIOLES, P. MARTINOT, X.-K. TONG, E. HAMEL; Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: Background: Hypertension increases the risk of developing Alzheimer's disease (AD) with increasing age. Cohort studies have shown lower incidence and progression to AD in elderly patients treated with anti-hypertensive medications targeting the renin-angiotensin system. The mechanism initiating these benefits is unknown; yet, blockade of angiotensin II (AngII) type 1 receptors (AT1Rs) may favor conversion of AngII to angiotensin IV (AngIV). AngIV receptors (AT4Rs) have been implicated in cognitive performance and cerebrovascular dilatory function. Objective: We investigated whether chronic intracerebroventricular (icv) administration of AngIV could restore cerebrovascular and cognitive function in transgenic mice overexpressing the Swedish and Indiana mutations of the human amyloid precursor protein (APP mice). Methods: APP mice (4.5 months old) were administrated (icv, 1 month) artificial CSF or AngIV (~1.3 nmol/day, AT4R agonist), delivered using osmotic minipumps. At endpoint, a MWM was performed to assess spatial learning and memory. Neurovascular coupling response to whisker stimulation was measured using laser Doppler flowmetry and cerebrovascular vasodilatory function was measured in segments of isolated and pressurized posterior cerebral arteries. Blood pressure was monitored by non-invasive tail-cuff plethysmography. Results: Chronic AngIV administration rescued neurovascular coupling and dilatations of cerebral arteries to acetylcholine, calcitonin gene-related peptide, ATP-sensitive potassium channel opener levcromakalim and transient receptor potential vanilloid 4 channel opener GSK1016790A, as well as baseline production of nitric oxide measured by incubating vessel segments with N^{ω}-nitro-$_{L}$-arginine. The impaired spatial learning capacity of APP mice was not

rescued following 1 month of AngIV delivery. However, memory retention was improved for the time and distance swam within the target quadrant despite a non-significant number of crossings over the hidden platform, suggesting a deficit with precision. Blood pressure was comparable in all groups. Conclusions: The AngIV/AT4R cascade rescued impairments in neurovascular coupling, cerebrovascular dilatory function and memory retention in APP mice. Our findings identify the AngIV/AT4R cascade as a promising target for restoring cerebrovascular deficits in AD.

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Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA I21BX002215

Cure Alzheimer's Fund

Title: Induced pluripotent stem cell differentiated three dimensional alzheimer's human neuronal culture exhibits compounds' efficacies on abeta production and tau phosphorylation

Authors: H.-K. LEE[1,4], C. VELAZQUEZ[1,5], M. CHEN[1,7], P. MORIN[1,6], *J. M. WELLS[2,3,6], E. HANLON[3], W. XIA[1,5];
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Abstract: Amyloid containing neuritic plaques and Tau containing neurofibrillary tangles are characteristic of the neuropathology that appears in the brains of Alzheimer disease (AD) patients. By combining two innovative technologies, induced pluripotent stem cells (iPSCs) and three-dimensional (3D) human neuronal culture, we have created cellular model systems representing five individual AD patients. We developed iPSCs from human peripheral blood mononuclear cells taken from each patient and differentiated these iPSCs into 3D human neuronal cultures. We validated the differentiation status of our 3D neurons by immunocytochemical staining to characterize neuronal markers. We treated these 3D neurons

either with inhibitors targeting β- or γ-secretase, two enzymes that cleave amyloid precursor protein (APP) to generate amyloid β peptide (Aβ), or with inhibitors targeting glycogen synthase kinase 3β (GSK3β), which phosphorylates Tau at multiple sites. We confirmed the drug exposure of 3D neurons and quantified the levels of these inhibitors inside of neurons at the end of treatment by quantitative liquid chromatography/tándem mass spectrometry. The β- and γ-secretase inhibitors blocked generation of Aβ in cultured neurons derived from all five AD patients. However, the GSK3β inhibitor exhibited variation among 5 AD patients' neuronal lines. Specifically, GSK3β efficacy for blocking Tau phosphorylation at residues threonine 181 versus threonine 231 varied among the individual patients' neurons. Furthermore, a different class of GSK3β inhibitor also demonstrated the same pharmacological inhibition profile for Tau phosphorylation in our individual AD patient derived neuronal systems. In conclusion, our 3D human neuronal cultures, differentiated from iPSCs derived from individual AD patients, represents the most physiologically relevant model for testing drug efficacy in vitro.

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Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSFC 81471160

Title: TFEB-mediated cellular clearance as a potential therapy for Alzheimer`s disease

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Abstract: Autophyagy is the basic catabolic mechanism that involves cell degradation of unnecessary or dysfunctional cellular components through the actions of lysosomes. Its dysfunction has been implicated in multiple neurodegenerative diseases with abnormal protein aggregates. The transcription factor EB (TFEB) can activate the autophagy-lysosome pathway (ALP) through coordinated expression of autophagy and lysosomal target genes. It also plays crucial roles promoting cellular clearance through transcriptional activation of endocytosis and membrane repair and lysosomal exocytosis. Our data has shown a highly efficacious effect of TFEB in ameliorating pTau and NFT pathology in rTg4510 mutant Tau transgenic mice. In

addition, it leads to the improved synaptic plasticity and cognitive improvements in this mouse model. Thus, TFEB is an attractive therapeutic target due to its ability to simultaneously activate autophagy and lysosomal biogenesis, its activity can be modulated through kinase inhibition. So we hypothesize that activation of TFEB by novel kinase inhibitors will be effective in clearing pTau and NFT pathology and improves cognitive outcomes. We propose to identify new TFEB activators by performing high-throughput screening of selected small molecule libraries using a TFEB-specific luciferase reporter assay and test the effect of the inhibitors on TFEB activity and tau/NFT pathology in rTg4510 mouse model. Above all, our study holds promise for intervening protein aggregate pathologies in AD and other neurodegenerative diseases.

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Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Grant 101925

Title: Hypercaloric diet-induced metabolic syndrome increases white matter inflammation and cognitive deficits in a transgenic rat model of Alzheimer's disease

Authors: ***N. IVANOVA**, N. WEISHAUPT, S. WHITEHEAD, D. CECHETTO;
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Abstract: Alzheimer's disease (AD) is a debilitating progressive incurable neurodegenerative disease. Metabolic disorders including obesity, metabolic syndrome (MS) and type 2 diabetes represent a risk factor for dementia including AD later in life. Unhealthy lifestyle choices, particularly the intake of high-caloric Western diet rich in saturated fat and simple carbohydrates, is a common risk factor for the development of metabolic syndrome and is associated with incidence of clinical dementia. The existence of a common basis for these two pathologies raises the possibility of a contribution of metabolic disorders to the course of the dementia when these conditions are co-morbid, however, the underlying mechanisms of this interaction are yet to be elucidated. And this knowledge may be crucial for defining a new therapeutic target and, moreover, may point out a possible preventative strategy.
In this study we investigate the effect of diet-induced metabolic disturbances on neuropathology, specifically examining the consequences of its chronic course on neuroinflammation, and cognitive function in a transgenic rat model carrying a human β-amyloid precursor protein gene

with Swedish and Indiana mutations, implicated in early-onset AD.
Wild type with intact genotype and transgenic rats 8.5-9 month old were fed either a hypercaloric (HCD) or a control diet for 12 weeks. Diet consumption and caloric intake were measured twice a week throughout the experiment. Assessed physiological parameters included body weight, glucose and insulin levels, serum lipid profile and blood pressure. Cognitive function was assessed using Morris water maze. Immunohistochemistry was used to examine neuroinflammation including staining for markers of activated microglia and astrocytes.
Rats maintained on the HCD developed significant obesity, visceral adiposity, dyslipidemia and hyperinsulinemia, but did not become hypertensive. Glucose metabolism was altered only in wild type rats on the HCD. Memory consolidation was impaired in the co-morbid model of AD and MS. Immunohistochemistry has shown greater white matter neuroinflammation in the co-morbid model in comparison to MS alone. Our data suggests that neuroinflammation might be one of the key elements linking early brain pathology to the neurodegeneration observed in AD and indicates anti-inflammatory agents may be a potential treatment strategy.

Disclosures: **N. Ivanova:** None. **N. Weishaupt:** None. **S. Whitehead:** None. **D. Cechetto:** None.

Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH

VA

Title: Cathepsin B is a validated drug target for traumatic brain injury (TBI), Alzheimer's disease (AD), and related neurodegenerative brain disorders

Authors: ***V. Y. HOOK**[1], J. JACOBSEN[2], K. GRABSTEIN[3], M. KINDY[4], G. HOOK, 92037[5]; [1]Skaggs Sch. of Pharmacy, and Dept. Neurosciences, LA Jolla, CA; [2]AstraZeneca, Cambridge, MA; [3]Univ. of Washington, Seattle, WA; [4]Univ. of South Florida, Tampa, FL; [5]American Life Sci. Pharmaceuticals, La Jolla, CA

Abstract: There is an urgent, unmet need for new drug targets in neurodegenerative diseases. Animal models and clinical correlates show that the cysteine protease cathepsin B is such a target. Mice lacking the cathepsin B gene in traumatic brain injury (TBI), Alzheimer’s disease

(AD), ischemia (IS), chronic inflammatory pain (CIP), epilepsy (EP) and multiple sclerosis (MS) all have improved behavioral deficits, neuropathologies, or biomarkers relative to sufficient animals. Specifically, TBI of cathepsin B deficient mice results in less neuromotor dysfunction, brain tissue loss and neuronal cell death than that which occurs in wild-type (wt) animals. Transgenic AD mice expressing human APP containing the wt beta-secretase site sequence and London mutation develop memory deficits and brain amyloid plaque and deleting the cathepsin B gene in such animals reduces the memory deficits, amyloid plaque and neurotoxic brain amyloid-beta, including the pernicious pyroglutamate amyloid-beta. IS in a cathepsin B gene knockout mouse results in less brain injury and inflammatory TNF-alpha, IL-1beta, IL-10 cytokine production than occurs wt animals. In a CIP model, deleting the cathepsin B gene reduces chronic inflammatory pain and inflammatory cytokines IL-1beta and IL-18. Removing the cathepsin B gene in an EP mouse model reduces the brain neuronal cell death that occurs in cathepsin B sufficient EP mice. In a MS model, deleting the cathepsin B gene and that encoding cathepsin S, which is another cysteine protease, increases the age of onset, improves the clinical score and reduces spinal cord leukocyte infiltration. Increased cathepsin B levels also correlate with worse outcomes in patient groups. Plasma cathepsin B is elevated in polytrauma patients and higher levels correlate with increased organ failure and death. AD patients have higher brain and plasma cathepsin B levels than controls and higher plasma levels correlate with reduced cognitive function. Patients with the chronic inflammatory neurological diseases Guillain-Barre syndrome, chronic demyelinating polyneuropathy and MS have higher cerebrospinal fluid cathepsin B levels than controls. Cathepsin B expression is higher in spinal tissue from amyotrophic lateral sclerosis patients than in control patients. Cerebral aneurysum results in vascular wall cathepsin B levels that are much higher than normal vascular tissue. These data from a variety of neurodegenerative models and clinical correlates strongly support cathepsin B as a new drug target for many neurodegenerative diseases.

Disclosures: **V.Y. Hook:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ALSP. **J. Jacobsen:** A. Employment/Salary (full or part-time): AstraZeneca. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ALSP. **K. Grabstein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ALSP. **M. Kindy:** None. **G. Hook:** A. Employment/Salary (full or part-time): ALSP. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ALSP.

Poster

513. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Cure Alzheimer's Fund

Title: Small molecule APP processing inhibitors lower Aβ peptide levels via cKit signaling

Authors: *C.-D. CHEN[1], E. ZELDICH[1], K. CAMARA[3], L. E. BROWN[3], J. A. PORCO[3], C. R. ABRAHAM[1,2];
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Abstract: Alzheimer's disease (AD) is characterized by the accumulation of neurotoxic amyloid beta (Aβ) peptides, which are proteolytically derived fragments of the amyloid precursor protein (APP). Inhibiting Aβ production may reduce neurodegeneration and cognitive dysfunction associated with AD. Ample evidence suggests that dimerization of APP plays a role in Aβ production; however, the mechanism involved is not fully understood. We have previously used an APP-firefly luciferase enzyme complementation assay to conduct a high throughput screen for inhibitors of APP dimerization, and have identified a compound that inhibits APP dimerization and reduces Aβ levels. In the present study, we have identified a number of analogs with improved efficacy in reducing Aβ, and identified a lead compound, Y10. A kinase-profiling assay identified the cKit receptor tyrosine kinase as a possible Y10 target. To elucidate the precise mechanism involved, APP phosphorylation was examined by IP-western blotting. We found that Y10, in addition to inhibiting cKit phosphorylation increases APP phosphorylation on tyrosine residue Y743 (APP751 numbering). A second specific cKit inhibitor was also found to increase APP phosphorylation. Next, we investigated the effects of compounds that target cKit downstream signaling molecules on APP phosphorylation and Aβ reduction. Our results suggest that the cKit signaling pathway is involved in APP phosphorylation, surface localization, dimerization, and Aβ production. Thus, reduction of Aβ *via* regulation of APP phosphorylation should be considered as a novel therapeutic strategy for AD.

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Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Expression of the stress response regulatory protein Dual Leucine Zipper Kinase (DLK) during the development of the neuropathology in Alzheimer's disease

Authors: *V. BUGGIA-PREVOT, S. GOODWANI, C. CHAKRABORTY, R. SHIN, P. ACTON, W. RAY;
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Abstract: Patients affected by Alzheimer's disease (AD) develop amyloid plaques and neurofibrillary tangles long before cognitive deficits and neuronal loss can be measured in the brain. However the precise mechanism and pathways leading to the progressive yet ultimately massive neuronal death during AD remain elusive. Modulating such pathways to promote neuroprotection would be an attractive therapeutic strategy to slow the progression of AD and preserve patients' cognitive function. Activation of c-jun N-terminal kinase (JNK) pathway is a critical player in triggering neuronal death in response to acute neuronal stress and injury. The neuron-specific mixed lineage kinase member Dual Leucine Zipper kinase (DLK, *Map3k12*) is required for JNK pathway activation in response to acute neuronal damage such as axonal injury, excitotoxicity, and toxin-induced neurodegeneration. Despite evidence of the JNK pathway activation in slowly progressing degenerative condition, DLK expression in the CNS and its potential contribution to neuronal loss in AD hasn't been evaluated. In the present study, we examined DLK, the activated phosphorylated form of JNK (pJNK), and the target of JNK, p-c-Jun, by immunofluorescence at various ages in multiple models of AD (APP TTA, 5XFAD, rTg4510, p25/cdk5). In control brains, DLK is expressed exclusively in neurons throughout most brain regions with a predominant localization to axons. However, DLK localization is affected early in the pathological process and followed by JNK pathway activation in all AD models examined. Both DLK and p-JNK accumulates in dystrophic neurites surrounding amyloid deposits in the brain of both amyloid models tested, whereas in p25/cdk5 and rTg4510, DLK accumulates in damaged axons. We propose that DLK might play a critical role in triggering JNK-dependent neurodegeneration in AD in addition to its well-known role regulating the acute neuronal stress response.

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Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cullen Trust

Mitchell Center for Neurodegenerative Disease

Sealy Center for Vaccine Development

Title: Characterizing p53 oligomers in neurodegenerative disease

Authors: *K. FARMER[1], C. LASAGNA-REEVES[2], R. KAYED[1];
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Abstract: The homotetrameric tumor suppressor, p53, is known as the “guardian of the genome” due to its role as a master regulator of cell cycle control, apoptosis, and DNA repair. P53 is the most frequently mutated gene in cancer cells and its loss of function is associated with over 50% of cancers, but p53 also plays a substantial role in aging. An increase in p53 can lead to premature aging, the primary risk factor for the most prevalent neurodegenerative diseases. Impaired proteostasis is implicated as an etiological factor in a growing number of diseases, notably neurodegenerative disorders and recently, cancer. When proteostasis is dysregulated, proteins misfold, aggregate, lose biological function, and eventually form large fibrils that disrupt the normal physiological function of nearby cells. Our lab and others have shown that p53 can aggregate into oligomers and fibrils similarly to other prion-like proteins implicated in neurodegeneration. The extent to which p53 oligomerizes in cancer has been shown to be dependent on the p53 mutation. This suggests that specific alterations to p53 may be critical to its propensity to form potentially toxic aggregates. Mutant p53 aggregates have been shown to seed the aggregation of wildtype p53 and also its paralogs, p63 and p73. This has led to the more alarming notion that P53, like the aggregant proteins associated with neurodegeneration, may have prion-like properties. Thus, we hypothesized that p53 oligomers may play a critical role in neurodegenerative disease. We demonstrated that p53 oligomers are the most toxic species in basal cell carcinoma, similar to findings for other amyloid proteins. We then evaluated p53 aggregation status in Alzheimer’s disease (AD) for the first time using brain tissue from AD patients and mice overexpressing mutated amyloid precursor protein (Tg2576 mice) by immunohistochemistry using novel antibodies with conformational epitopes common to oligomers of aggregant proteins and specific to oligomeric tau, F11G3 and T22, respectively. Our findings suggest that P53 oligomers may play a role in the progression of both cancer and Alzheimer’s disease and may interact with other aggregant proteins in disease. These results may

have implications for a number of other neurodegenerative disorders. Thus, it will be critical to understand which P53 mutations increase oligomerization and whether they are associated with different neurological disorders. Further research is needed to determine whether p53 may provide a new route for treatment of AD.

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Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Fulbright Nehru Postdoctoral Fellowship

Title: PPP3CB catalytic isoform mediated signaling of Calcineurin is significantly altered in Alzheimer’s disease.

Authors: *M. THAKER[1], E. HUDRY[3], S. HOPP[2], B. T. HYMAN[4];
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Abstract: Calcineurin (CN), the only Calcium-dependent protein phosphatase, mediates diverse physiological processes including neuronal differentiation, axonal transport, long term depression (LTD), inflammation, and autophagy by dephosphorylating critical signaling proteins and transcription factors. The holoenzyme is composed of a catalytic and a regulatory subunit. Three catalytic (PPP3CA, PPP3CB, PPP3CC) and three regulatory (RCAN1, RCAN2, RCAN3) isoforms of CN are described. Accumulating evidence suggests that CN inhibition is a specific target for alleviating symptoms associated with early synaptic changes in Alzheimer’s disease (AD). However the mechanism of CN action remains poorly characterized in the brain. Moreover nothing is known about which catalytic and regulatory isoform reveal altered expression and signaling in AD. Therefore we developed a series of assays to examine the CN deregulation in AD by comparing its three catalytic isoforms PPP3CA, PPP3CB, PPP3CC and two regulatory isoforms RCAN1 and RCAN2 using immunohistochemical and biochemical assays. Cytosolic, nuclear and synaptoneurosomal fractions from the frontal cortex of AD brains were compared to age-gender matched control brains. Strikingly, among the three catalytic isoforms only the truncated and constitutively active form of PPP3CB was upregulated, while PPP3CC was downregulated and PPP3CA remained unchanged in AD brains. Both RCAN1 and RCAN2 expressions were reduced in AD brains. My data implies that increased activation of CN

in AD is due to both increased truncation of PPP3CB and decrease in endogenous CN inhibitors RCAN1 and RCAN2. Notably the altered expressions of catalytic subunit as well as the regulatory proteins were significant mainly in the cytosolic fractions in AD brains. Immunohistochemistry demonstrated that the catalytic isoforms exhibit differential sub-cellular localizations in human brain; PPP3CA and PPP3CB are cytoplasmic and PPP3CC is axonal while RCAN1/RCAN2 localize both in the cytoplasm and nucleus. Taken together, these data suggest that the catalytic isoforms PPP3CB and PPP3CC reveal altered cleavage and regulatory isoforms show altered expression affecting the overall activity of CN in AD brains which certainly is linked with the pathogenesis of AD.

Disclosures: **M. Thaker:** None. **E. Hudry:** None. **S. Hopp:** None. **B.T. Hyman:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.04/Y18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Mitchell Center for Neurodegenerative diseases

Title: Formation of toxic oligomeric assemblies of RNA-binding protein Musashi in Alzheimer’s disease and other tauopathies

Authors: ***U. SENGUPTA**[1], K. LIN[2], G. MINUESA[3], M. G. KHARAS[4], R. KAYED[1]; [1]Neurol., [2]Univ. of Texas Med. Br., Galveston, TX; [3]Sloan Kettering Inst., New York, TX; [4]Sloan Kettering Inst., New York, NY

Abstract: Alzheimer’s disease (AD) is the most common neurodegenerative disorder associated with structural and functional alterations of brain cells causing progressive deterioration of memory and other cognitive functions. While neurofibrillary tangles (NFT’s) and amyloid beta plaques are the classical pathological hallmarks of AD, existing findings suggest that an intermediate species in the aggregation process, oligomers, are the prime neurotoxic entities in this disease and other neurodegenerative diseases. Recent studies demonstrate that in addition to amyloid-β, tau and α-synuclein aggregation, AD pathology also exhibits aggregates of transactive response DNA binding protein (TDP-43) and some RNA-binding proteins. The two isoforms of MUSASHI (MSI1 and MSI2) are important proteins in the mammalian central nervous system (CNS), and have been studied in stem cells and in various cancers. Preliminary studies indicate that MUSASHI1 (MSI1) is ectopically present in lesion-bearing neurons in AD and other neurodegenerative tauopathies. Here we have investigated the expression and

accumulation of MUSASHI, as well as their possible interactions with other cellular proteins in neurodegenerative diseases. We have carried out biochemical and biophysical assays with recombinant MUSASHI and have also performed immunohistochemical (IHC) and biochemical analyses of post-mortem brain tissues from AD and other tauopathies using our conformation-based anti-oligomeric antibodies in combination with commercially available MSI1 and MSI2 antibodies.
Our data, for the first time, demonstrate that MUSASHI are present in an oligomeric state in AD brain compared to the control. Normally, MUSASHI is found to be monomeric, but higher concentration of these proteins may lead to their aggregation. More interestingly, we found nuclear localization of MUSASHI oligomers in the diseased brains. Despite being similar in function, MSI1 and MSI2 tended to aggregate and associate with other cellular proteins in different manner. MSI2 was found to associate with TDP43 and tau more evidently than MSI1.

Disclosures: **U. Sengupta:** None. **K. Lin:** None. **G. Minuesa:** None. **M.G. Kharas:** None. **R. Kayed:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.05/Z1

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Palmitoylation in neurodegeneration

Authors: *C. DIEZ ARDANUY;
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Abstract: Neuronal Ceroid Lipofuscinoses (NCLs) are a group of hereditary, neurodegenerative disorders characterized by the accumulation of autofluorescent storage material in neurons. Depending on the age of symptomatic onset, NCLs are broadly classified infantile, juvenile and adult. Mutations in the *DNAJC5* gene encoding Cysteine-String Protein alpha (CSPα) have been identified as the cause of autosomal-dominant adult-onset NCL (ANCL) [1]. The disease-causing mutations (L115R and ΔL116) occur within the cysteine-string domain, a region of the protein that is extensively palmitoylated. Palmitoylation is a post-translational modification consisting of the attachment of fatty acids to cysteine residues through a thioester bond. We previously described that the ANCL mutations cause CSPα to form SDS-resistant aggregates, which are induced and maintained by S-acylation[2]. This observation is interesting as protein aggregation is a common feature of different neurodegenerative disorders. Using different proteasomal and

lysosomal inhibitors, we have been able to identify the degradation pathway of the CSP protein. Moreover, in order to investigate the role of palmitoylation and the cysteine string domain in aggregation, we transfected PC12 cells with previously generated mutant CSP proteins. With that purpose, we have been able to identify the residues involved in the aggregation process. Thus, the present study aims to identify the potential mechanisms involved in aggregation of ANCL mutant CSPα proteins and to further investigate how disruption of CSPα might cause the disease. Having identified the role of the cysteine-string domain in aggregation and the cellular pathways that mediate degradation of CSPα and ANCL mutant, we currently focus our interest in the characterisation of the CSP aggregates in human brains from ANCL patients and other neurodegenerative diseases. 1. Nosková L, Stránecký V, Hartmannová H, et al. Mutations in DNAJC5, encoding cysteine-string protein alpha, cause autosomal-dominant adult-onset neuronal ceroid lipofuscinosis. *Am J Hum Genet*. 2011;89(2):241-252. doi:10.1016/j.ajhg.2011.07.003. 2. Greaves J, Lemonidis K, Gorleku O a, Cruchaga C, Grefen C, Chamberlain LH. Palmitoylation-induced aggregation of cysteine-string protein mutants that cause neuronal ceroid lipofuscinosis. *J Biol Chem*. 2012;287(44):37330-37339. doi:10.1074/jbc.M112.389098.

Disclosures: C. Diez Ardanuy: None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.06/Z2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Increased pro-nerve growth factor induces RhoA activation in PC12 cells similar to Alzheimer's disease

Authors: J. SUSTARICH[1], S. SUCHDEVA[1], M. SYCHEVA[2], *R. B. JEGANATHAN[2], G. THANGIAH[1];
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Abstract: Recently we have shown that the expression of pro-nerve growth factor (proNGF) was significantly increased and NGF level was decreased in Alzheimer's disease (AD) hippocampal samples. NGF regulates cell survival and differentiation by binding TrkA and $p75^{NTR}$ receptors. ProNGF is the precursor form of NGF, binds to $p75^{NTR}$ receptor and induces cell apoptosis. Here, we show that the PC12 cells stimulated with proNGF significantly enhanced the expression of $p75^{NTR}$ receptor. The proNGF stimulation also increased the activation of RhoA

kinase and JNK apoptotic pathway. Interestingly, the activation of RhoA kinase and phosphorylation of JNK was also found to be increased in post-mortem human AD hippocampus compared to control, which might be due to increased expression of proNGF and $p75^{NTR}$ receptor. The addition of RhoA kinase inhibitor Y27632 not only blocked the RhoA kinase activity but also reduced the expression of $p75^{NTR}$ receptor induced by proNGF in PC12 cells. RhoA kinase inhibitor Y27632 also inhibited the proNGF induced neuronal death by abrogating the activation of JNK. These results suggest that overexpression of proNGF in AD enhances activation of RhoA thereby leading to neuronal cell death. Supported by Alabama Agricultural Experiment Station funding to RJ and Faculty Grant-in-aid to GT.

Disclosures: **J. Sustarich:** None. **S. Suchdeva:** None. **M. Sycheva:** None. **R.B. Jeganathan:** None. **G. Thangiah:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.07/Z3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG048935

Title: Examination of dose-dependency on the effects of life-long voluntary exercise intervention on behavior in a mouse model of cerebral amyloid angiopathy

Authors: *L. S. ROBISON[1], D. L. POPESCU[1], S. I. BEIGELMAN[1], S. A. AMREIN[1], A. E. KUZMINA[1], D. A. LITUMA[1], W. LIU[1], S. M. FITZGERALD[1], S. SUBZWARI[1], F. XU[2], J. DAVIS[2], W. E. VAN NOSTRAND[2], J. K. ROBINSON[1];
[1]Psychology, [2]Neurosurg., Stony Brook Univ., Stony Brook, NY

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder for which pharmacological treatment has proven largely ineffective, resulting in interest in alternatives for prevention and treatment, particularly cardiovascular exercise. Cerebral amyloid angiopathy (CAA) is a neurological condition in which fibrillar amyloid is deposited around the cerebral vasculature. CAA is an important component of AD, predicting early cognitive deficits. The TgSwDI mouse is a well-characterized model of CAA, and due to the vascular nature of the pathology, is an ideal model to examine the effects of exercise on CAA symptomology. Therefore, we are investigating the dose-dependent effects of exercise on C57 WT and TgSwDI mice (n=10/group) split into four groups at 3-4 months of age: Sedentary, 1h, 3h, or 12h daily access to running wheel. Mice are run five days per week for 10 months, with behavioral testing

occurring prior to exercise, at the mid-point of exercise, and at the end of exercise treatment. An interesting qualitative difference in exercise groups emerged. While number of rotations (exercise volume) was greater in the 12h group, 1h and 3h mice took fewer breaks, producing increased training intensity. TgSwDI mice showed comparable rates of running to WT controls though consumed more food and weighed more than WT mice. Running dose-dependently reduced body weight in both genotypes despite increasing food intake. TgSwDI mice were also less active in the open field (OF) and radial arm maze (RAM). Interestingly, there was an inverse dose response (intensity versus volume) of exercise on activity levels in the OF and RAM, with 1h mice being most active, and this was consistent across genotypes. TgSwDI mice exhibited marginally anxiogenic behavior in the OF and light-dark box test (LDB). Running dose-dependently reduced anxiety-like behavior in several settings. TgSwDI mice were not impaired on object displacement or novel object recognition tasks and tasks involving social behavior. TgSwDI mice were marginally impaired on the rotarod, and exercise normalized this deficit. These findings suggest that the benefits of exercise may be dependent on exercise intensity and behavior measured, and that TgSwDI mice may be particularly sensitive to some of the beneficial effects of cardiovascular exercise.

Disclosures: **L.S. Robison:** None. **D.L. Popescu:** None. **S.I. Beigelman:** None. **S.A. Amrein:** None. **A.E. Kuzmina:** None. **D.A. Lituma:** None. **W. Liu:** None. **S.M. Fitzgerald:** None. **S. Subzwari:** None. **F. Xu:** None. **J. Davis:** None. **W.E. Van Nostrand:** None. **J.K. Robinson:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.08/Z4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Illinois Department of Health

SIU-SOM

Title: PI3K/AKT signaling and GLUT3 translocation are decreased in the hippocampus of old 3xTg mice

Authors: ***C. M. GRIFFITH**[1], A. A. SHARP[1,2], G. M. ROSE[1,2], L. P. REAGAN[3,4], X. YAN[5], P. R. PATRYLO[1,2];
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Affairs Med. Ctr., Columbia, SC; [5]Anat. and Neurobio., Central South Univ. Xiangya Sch. of Med., Changsha, China

Abstract: Alzheimer's disease (AD) is characterized by CNS hypometabolism, neuropathological symptoms (β-amyloid plaques and neurofibrillary tangles) and dementia. A recent study on mid-aged humans suggests that insulin resistance is associated with regional cerebral hypometabolism (Willette et al., 2015) and a growing body of literature links Alzheimer's disease (AD) to diabetes and insulin resistance, although the mechanisms involved are unclear. Recently, Calon et al. (2014) reported that the 3xTg mouse model of AD exhibits peripheral glucose intolerance and prior data suggest that this model exhibits CNS hypometabolism (Nicholson et al., 2010). Since the primary source of CNS insulin is from the periphery, we tested the hypothesis that alterations in peripheral insulin can translate into altered CNS insulin signaling in 3xTg mice. Further, since the translocation of both glucose transporter 3 and 4 (GLUT3 and GLUT4) into the plasma membrane can be stimulated by insulin (Grillo et al., 2009; Uemura and Greenlee, 2006) we proposed that GLUT translocation could be altered. This could contribute to the hippocampal hypometabolism seen in 3xTg mice. We examined both the MAPK/ERK and PI3K/AKT insulin signaling pathways using immunoblotting in the hippocampus of 3xTg mice at a time point when neuropathology is observed (20-24 months). We then used immunohistochemistry (IHC) to assess whether this change could occur in a cell type or regionally specific manner. Additionally, we used immunoblotting to examine hippocampal GLUT3 and GLUT4 translocation by assessing GLUT levels in crude plasma membrane and total cell fractions. In aged 3xTg mice pAKT levels ($n = 7$ wild type, 8 3xTg; $p < 0.0001$) but not activated MAPK ($n = 8, 8$), are significantly decreased relative to age matched controls and IHC reveals that this change occurs throughout the hippocampus; astrocytes, pyramidal neurons and inhibitory neurons exhibited decreased pAKT immunoreactivity ($n = 4, 4$). Further, the level of GLUT3, but not GLUT4, was significantly decreased in the crude plasma membrane fraction of 3xTg mice ($n = 14, 14$; $p < 0.0001$); total GLUT levels were not altered. These data suggest that insulin signaling via the PI3K/AKT pathway can be altered in aged 3xTg mice and is associated with a decrease in GLUT3 translocation. This decrease in GLUT3 translocation could cause decreased glucose uptake in the CNS thereby contributing to AD-related hypometabolism and may explain why intranasal insulin administration can affect cognitive function and AD-related CNS hypometabolism.

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Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.09/Z5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Wexner Medical Research Fund

Ingram Autism Research Fund

Title: Human neural organoids: Predictive and personalized medicine models of brain diseases

Authors: *R. ANAND[1,3], S. B. MCKAY[2,3];
[1]Dept Pharmacol, [2]Dept Biol. Chem. and Pharmacol., Ohio State Univ., Columbus, OH;
[3]NEURXSTEM, Columbus, OH

Abstract: We engineered neural organoids (containing the retina, cortex, midbrain, hindbrain, brain stem and spinal cord) from normal as well as Alzheimer's disease (APP gene duplication) and tuberous sclerosis (TSC2Arg1743Gln) patient skin cells. Methods: We used transcriptomics, immunohistochemistry, and 3D whole brain imaging. Results: Transcriptomic results remarkably show comprehensive and accurate correlation of the dysregulated expression of hundreds of genes previously correlated with the clinical symptoms and/or pathologies of both of these diseases. For Alzheimer's disease these include genes for lipid homeostasis, inflammation, metal ion homeostasis, water homeostasis and longevity. For tuberous sclerosis these include genes for tumor formation, autism, blood pressure regulation, Zn++ ion homeostasis, Pb++ ion toxicity, round worm infections, and cholesterol metabolism among others. Discussion: Our results suggest that synchronous dysregulation of the spatial-temporal coordination of these genes cause these diseases. These genes possibly define disease-specific functional "homunculi" within the structure of the human genome.

Disclosures: **R. Anand:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NEURXSTEM LLC. **S.B. McKay:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NEURXSTEM LLC.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.10/Z6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NHMRC Project Grant APP1042889

ARUK-PhD2014-19

NHMRC Training Fellowship GNT568890

Title: Hippocampal neuron loss and hippocampal sclerosis in the population

Authors: *S. R. HOKKANEN[1], S. HUNTER[1], T. S. MINETT[1], H. A. D. KEAGE[2], F. E. MATTHEWS[3], T. M. POLVIKOSKI[4], C. BRAYNE[1];
[1]Univ. of Cambridge, Inst. of Publ. Hlth., Cambridge, United Kingdom; [2]Univ. of South Australia, Cognitive Neurosci. Lab. Sch. of Psychology, Social Work and Social Policy, Adelaide, Australia; [3]Newcastle University, Inst. for Hlth. and Society, Newcastle upon Tyne, United Kingdom; [4]Newcastle University, Inst. of Neurosci., Newcastle upon Tyne, United Kingdom

Abstract: *Introduction*: Hippocampal neuron loss is a common neuropathological finding in the aging brain. Old-age hippocampal sclerosis (HS) is a dementing disorder, characterised by severe neuron loss and gliosis in hippocampal CA1. However, there are currently no objective HS criteria, and diagnoses vary considerably. HS aetiology is unclear, although it has been associated with both ischemia and TAR-DNA-binding protein-43 (TDP-43)- related neurodegeneration.
Methods: Hippocampal sections (6-9µm thick) from 691 brains donated for the population-based cohorts Cambridge City over-75s Cohort or MRC Cognitive Function and Ageing Study were evaluated. A semi-quantitative protocol capturing severity and extent of hippocampal neuron loss independent of the underlying cause was developed using hematoxylin-eosin stained slides. Vascular pathology was noted. HS was first evaluated independently from the protocol, and then described by observed neuron loss patterns. 624/ 691 cases had pTDP-43 staining. TDP-43 solid neuronal inclusions and neurites were assessed semi-quantitatively by hippocampal area and entorhinal cortex layer. All scorings were inter-rater evaluated.
Results: Focal CA1 neuron loss bordering CA2 was significantly associated with vascular aetiology (χ^2=8.2, p=0.004), whereas neuron loss in the subicular CA1 end presented significantly more frequently with TDP-43 inclusions (z=4.21, p<0.001). 34 HS cases exhibited maximal five pyramidal neurons in each of over half CA1 fields-of-view at x200 magnification, and were not associated with vascular lesions. All HS cases presented with hippocampal TDP-43

pathology.
Conclusion: We propose a protocol to describe hippocampal neuron loss patterns, and show that there are distinct neuron loss patterns associated with different etiologies within the hippocampal CA1 in the elderly population. We suggest easily applicable objective semi-quantitative neuropathological criteria defining old-age HS.

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Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.11/Z7

Topic: C.02. Alzheimer's Disease and Other Dementias

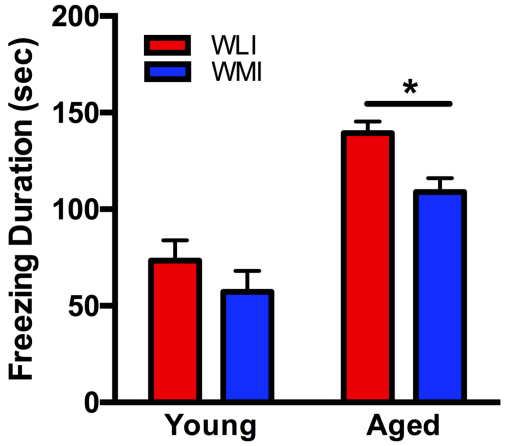
Support: Davee Foundation

Title: Mechanisms linking depression and dementia in a genetic rat model of depression

Authors: *P. LIM, S. L. WERT, E. TUNC-OZCAN, E. E. REDEI;
Psychiatry and Behavioral Sci., Northwestern Univ., Chicago, IL

Abstract: As the population ages, age related diseases such as dementia including Alzheimer's Disease (AD) will have an increasingly significant burden on the sufferers, their family and the society. Dementia, specifically AD, has been found to be associated with depression. Mid-life depression is a risk factor for late-life dementia, but the mechanism(s) linking depression and dementia are not known. Both AD and depression show greater prevalence in women. To investigate the molecular mechanisms common to some forms of depression and dementia, a genetic rat model of depression was used. The Wistar-Kyoto (WKY) rat strain has been established as an animal model for adult and adolescent depression due to their behavioral, hormonal and sleep characteristics. Selective breeding of WKYs resulted in two inbred strains. The more immobile WKY (WMI) rats consistently display depression-like behavior in the forced swim test compared to the control less immobile (WLI) strain. We tested young, 5-7 month old, and "middle-aged" 11-13 months old female WLIs and WMIs in hippocampus dependent contextual fear conditioning (CFC). The young females did not differ in their memory functions between the two strains. In contrast, the aged female WMIs showed fear memory deficits compared to their same age WLI controls. The enclosed figure shows the freezing duration during the second day of the CFC. Please note that normalizing day 2 freeze duration to day 1 eliminated the differences in freeze duration between the young and aged females, but retained

the significant difference between aged WLIs and WMIs. Hippocampal expression of genes known to be associated with dementia such as Presenelin-1, Presenelin-2, and Insulin-like growth factor 2, parallel these differences in fear memory. Hippocampal expression of memory loss- related genes could be used to further identify depression-related cause(s) of dementia. Understanding the underlying processes by which depression predisposes to later life memory loss may lead to prevention and perhaps treatment.



Disclosures: **P. Lim:** None. **S.L. Wert:** None. **E. Tunc-Ozcan:** None. **E.E. Redei:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH-NINDS grant 1SC1NS066988

NIH-NINDS 8R25NS080687

NIH-NIGMS 5R25GM061151

Title: EFhd2, a novel amyloid protein linked to neurodegeneration

Authors: *I. E. VEGA;
Translational Sciene and Mol. Med., Michigan State Univ. Clin. and Translational Sci. Inst., Grand Rapids, MI

Abstract: EFhd2 is a novel amyloid and calcium binding protein that has been associated with different neurological disorders. EFhd2 is highly expressed in neurons compared to other cell types of the central nervous system. The physiological function of this novel protein is still unclear, but it has been shown *in vitro* that may play an important role in synapse formation. EFhd2 co- localized with neurite markers such as tau, MAP2, synapsin and PSD95, suggesting that its neuronal function could be associated with vesicle transport and synapse homeostasis. Knockdown of EFhd2 increased synpasin 1a/b puncta labeling in neurites, suggesting that modulation of EFhd2 affects the development of functional synapses, but it had no effect on converting them to mature synapses as determined by the co-localization of synapsin and PSD95. Previous work showed altered expression of EFhd2 in Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, and schizophrenia, indicating that *Efhd2* gene expression could be modulated in response to neuropathological processes. Importantly, we showed that EFhd2 self-oligomerize and co-purify with filamentous tau proteins extracted from AD brain. However, the specific role that EFhd2 plays in the pathophysiology of neurological disorders is still poorly understood. Recent studies demonstrated that EFhd2 has structural characteristics similar to amyloid proteins found in neurological disorders. In order to dissect the molecular requirements that mediate EFhd2 oligomerization, we used a cell model system to question the role of phosphorylation, calcium binding and its coiled-coil domain in oligomerization. Additionally, we tested the effect that induced EFhd2 oligomerization has on cell survival. The understanding of EFhd2's oligomerization could lead to decipher molecular mechanisms that become activated in response to neuronal stress and degeneration.

Disclosures: I.E. Vega: None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.13/Z9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Chow Tai Fook Charity Foundation

Henderson Warmth Foundation

Title: Endothelin-1 induces formation of cofilin rods in hippocampal neurons via endothelin receptor type B and oxidative stress

Authors: *S. TAM[1], W. LAU[1], P. YEUNG[2], S. CHUNG[2], A. LAW[1];
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Abstract: Endothelin-1 (ET-1), which is a potent vasoconstrictor and neuromodulator, is shown to be over-expressed in the temporal lobe and white matter of post-mortem brains of patients who suffered from Alzheimer's disease (AD). In AD mouse models, ET-1 is also over-expressed in brain and may contribute to beta-amyloid (Aβ)-induced oxidative stress via binding to its receptors. On the other hand, oxidative stress induces cofilin to undergo dimerization and formation of rod-shaped aggregates, which contribute to loss of synapse and neuronal degeneration. In addition, an increase in cofilin rods can be seen in Aβ-treated *ex vivo* hippocampal slices or primary hippocampal neurons. Here, we hypothesized that the ET-1, of which level is increased in AD brain, may mediate cofilin rod formation via oxidative stress pathway in primary hippocampal neurons of mice.
Primary hippocampal neurons were isolated from C57BL/6N mouse on embryonic day 16 and cultured. In addition, these primary neurons expressed both ET receptor type A (ETAR) and type B (ETBR) on 14 day *in vitro* (DIV). On 14 DIV, neurons were treated with 100nM ET-1 with or without pretreatment of ETAR antagonist (BQ123) or ETBR antagonist (BQ788) for 24 hours. The involvement of oxidative stress in ET-1-induced rod formation was tested by co-treating neurons with ET-1 and N-acetylcysteine (NAC), which is known to block oxidative stress. Cofilin rods were visualized by immunostaining using anti-total cofilin antibody. The ET-1-induced oxidative stress was examined by CellRox reagent.
After 24h treatment with ET-1, there was a significantly higher percentage of neurons with cofilin rods, which was abolished by the co-treatment of NAC, suggesting ET-1 induces rod formation via oxidative stress pathway. In addition, ET-1-induced rod formation was also prevented by the pretreatment of BQ788, but not by BQ123, indicating the activation of ETBR pathway mediates the rod formation. Taken together, the over-expression of ET-1 in AD condition may induce oxidative stress-mediated cofilin rod formation.

Disclosures: **S. Tam:** None. **W. Lau:** None. **P. Yeung:** None. **S. Chung:** None. **A. Law:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.14/Z10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: microRNA profiling of human PSP, CBD, and DLBD brain tissues compared to normal controls

Authors: H. ESTRELLA[1], R. SORIANO[1], K. FISCHER[1], J. FRIEDMAN[2], D. DICKSON[3], *A. PAVLICEK[1];

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Abstract: Progressive Supranuclear Palsy (PSP) and Corticobasal Degeneration (CBD) are sporadic parkinsonian disorders with Tau pathology that share many molecular and clinical features. Patients with these disorders experience relentless neurodegeneration and typically succumb within 5 years of diagnosis. There are currently no disease modifying therapies for PSP or CBD, representing a significant unmet medical need.
In this study, we performed comprehensive microRNA profiling in brain samples from PSP (n=10) and CBD (n=10) independent human patients. As control groups, we used brain samples from patients with no neurodegenerative disease (n=10). We also included samples from patients with Diffuse Lewy Body Disease (DLBD) that lack Tau involvement (n=10). From each subject, the frontal cortex and cerebellum were isolated (total 80 samples) and profiled for microRNA expression using the Human Nanostring microRNA assays version 2. Data were normalized by positive controls and total microRNA signal, background adjusted, and batch adjusted using *ComBat* (R library *sva*). microRNAs with log2 expression > 2 in less than 10% of samples were removed from analysis (low expression). 461 sufficiently expressed microRNAs passed this cut-off and were used in subsequent differential expression analyses.
Many microRNAs were differentially expressed in PSP, CBD, and DLBD patients compared to controls. Expression changes in the frontal cortex were much more pronounced compared to the cerebellum. In the cortex, we found 54 microRNAs commonly deregulated at a false discovery rate (FDR) < 20% in both PSP and CBD samples compared to normal controls. Most of the significant microRNAs, including many brain-specific transcripts, were downregulated indicating potential cell loss. A similar pattern of down regulation was observed in the cortex of DLBD samples compared to normal controls. Many of the differentially expressed microRNAs identified in this study have been implicated in pathogenesis of other neurodegenerative diseases and may represent common drivers of neurodegenerative disorders warranting further investigations.

Disclosures: **H. Estrella:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **R. Soriano:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc. **K. Fischer:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc.. **J. Friedman:** None. **D. Dickson:** None. **A. Pavlicek:** A. Employment/Salary (full or part-time): Regulus Therapeutics, Inc..

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.15/Z11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Impaired wnt signaling in the prefrontal cortex of alzheimer's disease patients

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Abstract: Objective: To investigate the transcriptional and translational link between the Wnt signaling and Alzheimer's disease (AD) pathology in the prefrontal cortex.
Background: AD is a progressive neurodegenerative brain disorder and is the leading cause of dementia in the elderly population. AD is accompanied by three main pathological changes: diffuse loss of neurons, intracellular protein deposits termed neurofibrillary tangles and extracellular protein deposits termed neuritic plaques. The Wnt singaling pathway comprises an evolutionary conserved family of proteins important during embryonic neural patterning and the regulation of cell proliferation, polarity and fate determination. The canonical Wnt signaling negatively regulates the protein glycogen synthase kinase-3 beta (GSK-3β) activity. It has been suggested that increased GSK-3β activity is linked to both "genetic" and "sporadic" forms of AD, suggesting a crucial role of GSK-3β in AD pathogenesis. However, little is known about the specific properties of GSK-3β in AD.
Methods: Using microarray analyses, gene expression profiles were investigated in post-mortem brain tissue from patients (*n*=30) with AD and age-matched controls (*n*=30) in three interconnected areas of the prefrontal lobe structures. Furthermore, the significant findings in the gene expression were validated using RT-qPCR and Western Blotting.
Results: Our data indicate that in AD several genes related to the Wnt pathway are aberrant on both transcriptional and post-/translational levels. Noteworthy, we observed a switch in the dynamics of the two main components of the pathway, the transcriptional activator, β-catenin, and the regulatory GSK-3β kinase in the orbitofrontal cortex and medial frontal gyrus, whereas the superior frontal gyrus showed only modest alterations. Furthermore, we found that the aberrancy was correlated to the disease progression following post-mortem Braak Stage evaluation.
Conclusions: Our study is the first to comprehensively evaluate the Wnt signaling pathway in the prefrontal cortex of AD brains. At the transcriptional level, several Wnt signaling related genes in AD patients were found to be altered compared to healthy controls. Furthermore, we found that the two main components in the Wnt signaling pathway were altered either in activity or on protein level, and was in most cases correlated to the disease progression. Overall our findings suggest that increased GSK-3β activity is associated with AD pathogenesis and could potentially act as a therapeutic target.

Disclosures: J. Folke: None. **B. Pakkenberg:** None. **T. Brudek:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Program#/Poster#: 514.16/Z12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HL 123331

HL 124576

HL 079588

Title: Chronic short sleep fuels embers of Alzheimer's in locus coeruleus neurons

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Abstract: Chronic short sleep (CSS) is pervasive in modern societies; yet recent studies raise the possibility that sleep loss might exacerbate Alzheimer's disease (AD). AD animal model studies demonstrating increased plaque burden and greater neurobehavioral impairment have all been performed using transgenic amyloid precursor protein (APP) models where APP is massively upregulated. We have shown that CSS in the adult mouse results in mitochondrial metabolic stress and degeneration of locus coeruleus neurons (LCn). Intriguingly, LCn are highly susceptible to early deposition of tau aggregates and degeneration and may contribute to the spread of tau. We hypothesized that CSS increases LCn APP processing to Aβ, that in turn disturbs sirtuin 3 deacetylase activity and increases acetylation of tau within the LCn that over time could spread. Adult amyloid precursor protein knock-in mice with the Swedish mutation ($APP_{KI}NL$) and wild type (WT) controls were randomized to CSS or rested conditions for 4wk and then allowed to recover for 4wk. Rested APP_{KI} mice relative to WT, showed reduced LCn counts (25%). Both genotypes showed comparable LCn loss upon CSS (25%). $APP_{KI}NL$ exposed to CSS showed greater mitochondrial lysine acetylation and reduced SirT3 levels. Additionally APP mRNA increased significantly in both WT and $APP_{KI}NL$ exposed to CSS and amyloid beta in LC micropunches showed increased oligomerization. Importantly both WT and $APP_{KI}NL$ mice exposed to CSS showed impaired spatial object recognition. Collectively, these findings support that CSS markedly alters APP levels and modifications within LCn, changes that persist beyond sleep loss and that CSS has important molecular and behavioral effects in not only the $APP_{KI}NL$ mouse but WT, as well. Remarkably, changes in mitochondrial homeostasis, amyloid processing and LC degeneration happen in WT mice, suggesting a key role for sleep loss in sporadic AD.

Disclosures: **S.C. Veasey:** None. **Y. Zhu:** None. **P. Fenik:** None. **G. Zhan:** None. **Z. Zhao:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association NIRG-11-204995

NIG Grant R01NS083704

Title: Deficiency of membrane-anchored CX3CL1 lead to reduced cell-surface expression of CX3CR1 and elevates tau pathology in a model of systemic inflammation.

Authors: N. MAPHIS[1], G. XU[3], O. N. KOKIKO-COCHRAN[4], S. JUNG[5], J. CANNON[1], R. RANSOHOFF[6], B. T. LAMB[3], *K. BHASKAR[2];
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Abstract: One of the primary means of neuron-microglia communication occurs via secretion of a neuronally derived chemokine, fractalkine (FKN) or CX3CL1, which binds to its cognate receptor (CX3CR1) on microglia and in turn regulates microglial function. FKN communicates with CX3CR1 either as a membrane-anchored ligand or as a soluble molecule following constitutive cleavage by proteases. Our previous studies have suggested that the CX3CR1 deficiency in microglia lead to accelerated tau pathology and cognitive impairment in an hTau mouse model of tauopathy. We also observed elevated tau pathology within the brains of non-transgenic mice when they received reactive microglia derived from hTau*Cx3cr1*$^{-/-}$ donor mice. However, it is still unclear whether the soluble versus membrane-anchored form of FKN is responsible for the negative regulation of microglial activation. Here we utilized a recently characterized transgenic mouse model (FKN$^{\Delta 105}$/mFKN$^{-/-}$ mice) which expresses only obligatory soluble FKN (FKN$^{\Delta 105}$) in the mouse FKN knockout background (mFKN$^{-/-}$) and studied the effects of inflammation via LPS-induced tau phosphorylation. First, the basal level of phosphorylated tau (pTau) on the AT8 (S202) site was higher in mFKN$^{-/-}$ and FKN$^{\Delta 105}$/mFKN$^{-/-}$ mice compared to non-transgenic and mFKN$^{+/-}$ mice. Second, the level of pTau on the AT180 (T231) site was lower in mFKN$^{+/-}$ mice compared to non-transgenic mice. Interestingly, LPS administration did not induce any alterations in the pTau levels except there was a decreased

trend in both $mFKN^{+/-}$ and $mFKN^{-/-}$ mice at both the AT8 and AT180 sites. Third, mononuclear cells isolated from the $FKN^{\Delta 105}/mFKN^{-/-}$ mice showed a significant reduction in the expression of CX3CR1 compared to $mFKN^{-/-}$ mice. Finally, $FKN^{\Delta 105}/mFKN^{-/-}$ mice showed significant behavioral impairments in the Elevated Plus Maze and Novel Object Recognition behavioral tasks. Together, these results suggest that exclusive expression of the soluble form of FKN by neurons renders microglia unable to suppress neuroinflammation because of the reduced CX3CR1 expression on the cell surface and results in elevated tau hyperphosphorylation.

Disclosures: **N. Maphis:** None. **G. Xu:** None. **O.N. Kokiko-Cochran:** None. **S. Jung:** None. **J. Cannon:** None. **R. Ransohoff:** None. **B.T. Lamb:** None. **K. Bhaskar:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS76896

Title: The biophysical determinants of prion neuroinvasion

Authors: ***C. SIGURDSON**[1,3], J. LAWRENCE[2], C. BETT[2], T. KURT[2], C. ORRU[4], P. AGUILAR-CALVO[2], C. WU[2], B. CAUGHEY[5];
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Abstract: Pathologic beta-sheet rich protein aggregates accumulate in several devastating neurodegenerative diseases, including Alzheimer’s, Parkinson’s, and prion disease. Yet only prion protein aggregates have the unique ability to spread from extraneural sites into the interstitial spaces of the brain, causing fatal neurodegenerative disease. Interestingly, rare prion subtypes replicate extraneurally without spreading into the brain, leading to persistent subclinical carriers, and the factors that underlie the transfer of prion aggregates into the brain are unclear. Using prion strains having the same sequence but differing in structure, we identify a subset with a limited capacity for neuroinvasion in vivo. These prion aggregates rarely invade the CNS following intraperitoneal or intra-tongue inoculation, yet accumulate in extraneural organs, leading to lifelong subclinical carriers of infectious prions. Intriguingly, these poorly neuroinvasive prions are fibrillar, which are uncommon structures in prion disease, as most prions form oligomers. In vitro, however, these poorly invasive prions are endocytosed and can

spread from the axon terminal to the neuronal cell body. We found that reducing the prion aggregate size markedly impacts the neuroinvasive ability of fibrillar prions, suggesting that size plays an important role in prion neuroinvasion. Additionally, we have found that the intravenous route is surprisingly efficient for fibrillar prion entry into the CNS, which may help explain how human to human transmission of variant-Creutzfeldt Jakob disease prions occurred following blood transfusion. Taken together, these results indicate that aggregate size and route of exposure are major determinants that impact the ability of a prion to neuroinvade and may help explain how prions, which are largely oligomeric, spread so effectively as infectious pathogens.

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Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Neurodegeneration-like pathological and behavioral changes in an AAV9-mediated p25 overexpression mouse model

Authors: ***Y. HU**, X. ZHOU, J. HUANG, S. PAN, M. XU, R. HE, Z. JI;
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Abstract: Background: The transgenic mice models overexpressing human p25 contribute greatly to the in-vivo neurotoxic mechanism of p25 in neurodegenerative diseases. However, it is time-consuming to manipulate existing transgenic mice models. Objective: Here, we aim to establish a novel mouse model of neurodegeneration by overexpressing p25 mediated by recombinant adeno-associated virus serotype 9 (rAAV9). Methods: AAV9-GFP-p25 encoding GFP-fused p25 driven by synapsin promoter, and the control, AAV9-GFP, were delivered in mice by tail-vein injection. Assessments of p25 expression, neurodegenerative pathology, and behavioral changes were performed. Results: GFP expression was detected by in-vivo imaging as early as one week after virus injection. Notably, widespread expression of p25 was obviously

found in cortex, hippocampus and cerebellum in AAV9-GFP-p25 mice. Moreover, decreased hippocampus volumes in AAV9-GFP-p25 mice were detected by 7T MRI examination about one month after injection. Further, these AAV9-GFP-p25 mice exhibited progressive memory impairment from three-month to six-month after virus injection. At last, hyper-phosphorylated tau, neurofibrillary tangles, activated astrocytes and microglia cells were elevated in these p25 mice at about six months after virus delivery. However, amyloid-β (Aβ) plaque, overt neuronal loss and apoptosis in the hippocampus and cortex were not significantly induced by AAV9-mediated p25 overexpression. Conclusion: The AAV9-mediated p25 overexpression mouse model, which is a practical model exhibiting neurodegeneration-like pathological and behavioral changes, provides an easier and time-saving method to explore the functions of p25 in vivo, as well as an alternative tool for development of drugs against neurotoxic of p25.

Disclosures: **Y. Hu:** None. **X. Zhou:** None. **J. Huang:** None. **S. Pan:** None. **M. Xu:** None. **R. He:** None. **Z. Ji:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Support: Alzheimer’s Association MNIRGDP-12-258900 (CAH)

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Linda Crnic Institute (CAH)

NIH R01 NS086933-02 (CAH)

Title: RCAN1 overexpression and mitochondrial dysfunction

Authors: *H. WONG, J. LEVENGA, C. ARDIZZONE, C. HOEFFER;
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Abstract: Patients with Down syndrome (DS) develop symptoms consistent with early onset Alzheimer’s disease (AD), suggesting that factors involved in DS may also play a role in AD onset. DS is caused by trisomy of all or part of chromosome 21, resulting in overexpression of

genes within this region. One such gene is *Regulator of calcineurin 1* (*RCAN1*). RCAN1 is a potent regulator of the calcium/calmodulin-dependent phosphatase calcineurin and is required for long-lasting memory and synaptic plasticity. Overexpression of RCAN1 has been observed in brain tissue from not only DS patients but also AD patients and with normal aging. We recently found that brain-specific overexpression of a human RCAN1 isoform in mice promotes early age-dependent memory and synaptic plasticity deficits, tau pathology, and dysregulation of dynamin-related protein 1 (DRP1) activity associated with mitochondrial dysfunction and oxidative stress, reproducing key AD features. We are currently investigating how RCAN1 overexpression may alter calcineurin signaling to promote these phenotypes. In addition, we are investigating how increased RCAN1 levels may contribute to AD-related pathology in DS using the *Dp(16)1Yey/+ (Dp16)* mouse model of DS. These studies should provide insight into the molecular basis of AD progression in DS and the general population and whether RCAN1 is a therapeutic target for treatment.

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Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NINDS T32 NS077888

1RF1-AG051495

Title: CRISPR-Cas9-mediated knock-in of TREM2 R47H significantly modifies Alzheimer's disease pathology & neuroinflammation *In vivo*

Authors: *P. J.-W. CHENG-HATHAWAY[1], E. REED[1], T. R. JAY[1], S. BEMILLER[2], S. PUNTAMBEKAR[2], J. C. KARLO[1], G. XU[2], R. M. RANSOHOFF[3], G. E. LANDRETH[1], B. T. LAMB[2];
[1]Neurosciences, Case Western Reserve Univ. Sch. of Med., Cleveland, OH; [2]Neurosciences, Indiana Univ. Sch. of Med., Indianapolis, IN; [3]Neuroimmunology, Biogen, Boston, MA

Abstract: Variants in the gene encoding the Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) confer a greatly elevated risk for development of late onset Alzheimer’s disease (AD). The R47H missense mutation linked to AD risk was proposed to be a loss of function mutation. Recently, Jay et al., (2015) found that homozygous TREM2 knockout in the APPPS1 AD mouse

model resulted in significant attenuation of AD-like pathology and neuroinflammation at 4 months of age. Flow cytometry and bone marrow chimera studies suggested that plaque associated myeloid cells were infiltrating monocytes and that the survival and association of these cells with plaques was dependent on TREM2. Moreover, gene expression studies demonstrated that the loss of TREM2 resulted in a phenotypic shift to expression of anti-inflammatory genes. These data suggest that TREM2 loss of function ameliorates inflammatory processes driving AD pathology, a conclusion that is inconsistent with the postulated loss of function of the R47H TREM2 variant. To further examine the role of the R47H allele in AD pathogenesis, CRISPR/Cas9 genome editing technology was utilized to generate a *Trem2*$^{R47H/+}$ knock-in mouse and crossed to the APPPS1 mouse model. Immunohistochemical and biochemical assays were utilized to assess AD pathology and neuroinflammation in this novel model. Strikingly, *Trem2*$^{R47H/+}$; APPPS1 mice exhibited a decrease in amyloid plaques compared to *Trem2*$^{+/+}$; APPPS1 mice at 4 months as assessed by immunohistochemistry. Levels of amyloid precursor protein (APP) and the C-terminal fragment and amyloid beta generated by APP cleavage were significantly reduced. Finally, the number of plaque associated myeloid cells was found to be significantly decreased in the *Trem2*$^{R47H/+}$; APPPS1 mice. These preliminary results suggest that the TREM2 R47H mutation may confer both a gain- and loss-of-function mutation conferring significant differences in amyloid processing and neuroinflammation. Further experiments are needed to understand how the R47H TREM2 variant impacts myeloid cell function and will ultimately provide new insights into disease mechanisms and potential therapeutic targets for AD.

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Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS078363

NIH Grant T32 AG023477

Title: Contactins function as ligands for amyloid precursor proteins in regulating neuronal migration

Authors: J. M. RAMAKER[1], T. L. SWANSON[1], *P. F. COPENHAVER[2];
[1]Cell & Developmental Biol., [2]Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Although Amyloid Precursor Protein (APP) is the source of amyloid fragments that accumulate in Alzheimer's disease (AD), APP may also regulate neurogenesis and differentiation in the developing nervous system, albeit via mechanisms that are still controversial. Studies in cell culture suggested that APP can interact with the heterotrimeric G protein Goα and function as an unconventional G protein-coupled receptor, but compensatory interactions by other related proteins have hindered an analysis of this process in the mammalian brain. Using Manduca (hawkmoth) and Drosophila (fruit fly) as simpler models, we have shown that the sole insect ortholog of APP (APPL) directly binds Goα in the leading processes and synaptic terminals of developing neurons, and that endogenous APPL-Goα interactions are regulated by Goα activation. In cultured embryos, we found that stimulating APPL-Goα signaling restricts the polarized outgrowth and migration of developing neurons, consistent with other evidence that APP family proteins function as neuronal guidance receptors. Recent studies have shown that GPI-linked Contactins can interact with APP, potentially acting as binding partners or co-receptors. We have now shown that Manduca Contactin (MsContactin) is selectively expressed by glial cells that ensheath migratory neurons (expressing APPL), and that Contactin-APPL signaling regulates neuronal-glial adhesive interactions. Short-term treatment with Contactin-Fc fusion proteins labeled the migratory neurons in an APPL-dependent manner, while more prolonged treatment inhibited their migration and outgrowth, an effect that was blocked by knocking down APPL expression or preventing Goα activation. Conversely, APPL fusion proteins labeled the ensheathing glial cells, an interaction that was MsContactin-dependent. These results support the model that Contactins function as authentic ligands for APP family proteins in the developing nervous system, whereby Contactin-induced activation of APP-Goα signaling regulates neuronal growth and motility. Conversely, abnormal stimulation of this pathway might cause the aberrant activation of Goα and its downstream effectors, resulting in neurite retraction, synaptic loss, and neurodegeneration. In support of this model, APP-Goα interactions were reduced in brains from AD patients compared to healthy controls. We are currently investigating how this signaling pathway is altered in the aging brain, with the goal of understanding how the misregulation of APP-Goα signaling might contribute to the progressive neurodegeneration that typifies AD. Funding: NIH NS078363 (PFC); NIA T32 AG023477 (JMR).

Disclosures: J.M. Ramaker: None. **T.L. Swanson:** None. **P.F. Copenhaver:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R03 AR063326

Title: The mitochondria-targeted antioxidant MitoQ affects memory retention and neuropathology in aged 3xTgAD mice

Authors: *M. L. YOUNG, J. L. FRANKLIN;
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Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disorder. Despite extensive effort, there remains no effective therapy beyond symptomatic treatments. Considerable evidence suggests neuropathologies occurring well before the appearance of amyloid plaques and neurofibrillary tangles may have a role in disease progression. As oxidative stress is an early occurrence in neurodegeneration, we focus on this pathology as a potential mediator of AD. Dysfunctional mitochondria are a likely source of reactive species that contribute to oxidative stress. To further understand oxidative stress derived from mitochondria in the etiology of AD, we took advantage of a novel mitochondria-targeted antioxidant, mitoquinone mesylate (MitoQ). We've previously published data showing that MitoQ treatment in young 3xTg-AD mice, prior to pathology development, prevents oxidative stress, memory loss and several other AD-like pathologies present in this animal model. To determine whether this same antioxidant treatment would be effective during later disease progression we chose two time points to study. Mice began treatment at 7 and 12 months old and received MitoQ (100μM) continuously in drinking water for 5 months. At both age points, several AD-like disease hallmarks are present. Following treatment, mice underwent behavioral assessment and brain tissue was harvested for biochemical assays. Morris Water Maze training, a measure of spatial memory retention, showed that mice treated with MitoQ in each group learn spatial cues an average of 3 days before littermate controls. Interestingly, memory retention varied among age groups. In the younger age group, MitoQ treated animals and littermate controls retain memory equally in a measure of short- term memory retention. However, littermate controls did not retain spatial memory in long-term memory task. In our oldest group, mice treated with MitoQ retain spatial memory better than littermate controls in both short and long-term memory retention task. Sensorimotor deficiencies and escape motivation from the water maze were evaluated and did not prove to be significantly different between treatment groups or age groups. Supporting our behavioral data, MitoQ altered several AD-like pathologies in these mice. Synaptophysin, a marker for synapse loss, was significantly increased in MitoQ treated animals while nitrotyrosine

and reactive astrocytes were significantly reduced. Caspase-3 activity, implicated in tau pathology, and hyperphosphorylated tau were both significantly reduced with treatment. However Aβ 1-42 load remained the same in both treatment groups.

Disclosures: **M.L. Young:** None. **J.L. Franklin:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Consortium for FTD Research

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NIH F30AG046088

Glenn/AFAR postdoctoral fellowship

Title: Progranulin insufficiency drives biphasic social dominance abnormalities associated with altered neuronal morphology in the amygdala and prelimbic cortex

Authors: *E. D. ROBERSON, A. J. FILIANO, B. A. WARMUS, A. M. HALL, A. E. ARRANT;
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Abstract: Loss-of-function mutations in progranulin (*GRN*) are a major autosomal dominant cause of frontotemporal dementia (FTD), a neurodegenerative disorder in which social behavior is disrupted. As disease causing mutations are all loss-of-function mutations, progranulin haploinsufficiency is thought to cause FTD in patients with *GRN* mutations. Therefore, treatments that boost progranulin levels may be an effective strategy to prevent or treat FTD due to *GRN* mutations. Progranulin-insufficient mice ($Grn^{+/-}$) model the progranulin haploinsufficiency of FTD patients with *GRN* mutations and develop abnormal social behavior beginning around 6 months of age, making them a potentially useful model for preclinical testing. We have previously reported that $Grn^{+/-}$ mice develop increased social dominance in the tube test at 6 months of age. To assess the usefulness of this abnormal behavior for preclinical testing, we investigated how the tube test phenotype of $Grn^{+/-}$ mice changes with age,

determined its robustness under several testing conditions, and performed a proof-of-principle study for progranulin-boosting therapies by overexpressing progranulin in the brain with an AAV vector. We also explored cellular mechanisms associated with these tube test dominance abnormalities. We observed biphasic social dominance abnormalities in *Grn*$^{+/-}$ mice: at 6-8 months, *Grn*$^{+/-}$ mice were more dominant than wild-type littermates, while after 9 months of age, *Grn*$^{+/-}$ mice were less dominant. The dominant phenotype of 6-8 month-old *Grn*$^{+/-}$ mice diminished with repeated testing, while the low dominance phenotype of older *Grn*$^{+/-}$ mice was robust over many testing sessions. In a proof-of-principle study for progranulin-boosting therapies, we found that overexpressing progranulin with an AAV-progranulin vector reversed the low dominance phenotype of older *Grn*$^{+/-}$ mice. In studies of the underlying mechanisms of the biphasic tube test abnormalities of *Grn*$^{+/-}$ mice, we found associations of abnormal cellular signaling and neuronal morphology in the amygdala and prelimbic cortex with tube test behavior. At 6-9 months of age, when *Grn*$^{+/-}$ mice are dominant, we observed evidence of potentially elevated mTORC2 signaling in the amygdala, as well as enhanced dendritic arbors in the basomedial amygdala. At 9-16 months of age, when *Grn*$^{+/-}$ mice are submissive, we observed impaired basal dendritic arbors in the prelimbic cortex, a region which drives social dominance in the tube test. These data demonstrate that tube test abnormalities in *Grn*$^{+/-}$ mice are a useful preclinical model, and are associated with dysfunction in brain regions that also degenerate in FTD.

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Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Support: Consortium for Frontotemporal Dementia Research

American Federation for Aging Research Glenn/AFAR Postdoctoral Fellowship

NINDS Grant F32NS090678

Title: AAV-progranulin improves pathology in Grn$^{-/-}$ mice, an animal model of CLN11-neuronal ceroid lipofuscinosis (NCL)

Authors: *A. E. ARRANT, V. ONYILO, D. UNGER, E. D. ROBERSON;
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Abstract: Progranulin (*GRN*) is a secreted glycoprotein that modulates inflammation and is critical for normal lysosomal function. Loss-of-function *GRN* mutations are a major autosomal dominant cause of Frontotemporal Dementia (FTD), and individuals homozygous for loss-of-function *GRN* mutations develop the lysosomal storage disorder Neuronal Ceroid Lipofuscinosis (NCL). NCL is a devastating, early onset neurodegenerative disorder with multiple genetic subtypes that causes retinal degeneration and seizures. NCL due to *GRN* mutations has been designated as CLN11-NCL. *Grn*$^{-/-}$ mice develop retinal degeneration, thalamic hyperexcitability, and NCL-like pathology, thus providing an animal model of NCL due to progranulin deficiency. *Grn*$^{-/-}$ mice exhibit the characteristic NCL lesion, accumulation of autofluorescent lipofuscin granules, as well as astrogliosis and microgliosis throughout the brain. We hypothesized that restoring progranulin to *Grn*$^{-/-}$ mice might improve this NCL-like pathology. To test this hypothesis, we injected an AAV2/1 vector expressing mouse progranulin (AAV-*Grn*) into the medial prefrontal cortex (mPFC) of 10–12-month-old wild-type and *Grn*$^{-/-}$ mice. At this age, *Grn*$^{-/-}$ mice exhibit robust NCL-like pathology. Additional wild-type and *Grn*$^{-/-}$ mice were injected with AAV-GFP as a control, and uninjected *Grn*$^{-/-}$ mice were run to provide a baseline measure of pathology in the absence of AAV. All mice were euthanized 8–10 weeks after injection, and brains were processed for immunohistochemistry. AAV-*Grn* strongly overexpressed progranulin in wild-type and *Grn*$^{-/-}$ mice, such that AAV-*Grn*-treated *Grn*$^{-/-}$ mice had higher progranulin levels in the mPFC than AAV-GFP-treated wild-type mice. In support of our hypothesis, we observed significant reductions in lipofuscinosis as well as in microgliosis (CD68 immunoreactivity, Iba1+ cell morphology), but no change in astrogliosis (GFAP immunoreactivity) in regions distant from the injection site (motor cortex, CA3 of hippocampus, and ventral posterior thalamus). At the injection site in the mPFC of *Grn*$^{-/-}$ mice, we observed an increase in microgliosis with AAV-*Grn* (CD68 immunoreactivity), as well as immune activation (MHCII immunoreactivity). This indicates a local inflammatory reaction that could be driven by a non-self reaction to progranulin or cleavage of progranulin into pro-inflammatory granulin fragments. As this negative side effect is unlikely to occur in CLN11-NCL patients, who are not completely progranulin-deficient, these data support the use of progranulin-boosting therapies for CLN11-NCL.

Disclosures: **A.E. Arrant:** None. **V. Onyilo:** None. **D. Unger:** None. **E.D. Roberson:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.26/AA8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5T32AG020506-13

NIH Grant 5R25GM079300-07

Title: Progranulin and cathepsin D: A potential mechanism for neurodegeneration

Authors: *C. B. VALDEZ;
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Abstract: Frontotemporal Dementia (FTD) encompasses a group of neurodegenerative disorders characterized by cognitive and behavioral impairments as a result of progressive degeneration of frontal and temporal lobes. A mutation in the gene encoding progranulin (PGRN) accounts for up to 25 percent of familial FTD and results in decreased progranulin expression. Progranulin is normally expressed in neurons and microglia within the CNS but the function of progranulin and the mechanism by which its decrease leads to disease is still unknown. While progranulin has been implicated in a wide array of biological functions including inflammation and neurite outgrowth, recent literature has shown that complete loss of progranulin due to a homozygous PGRN mutation leads to neuronal ceroid lipofuscinosis (NCL), a group of neurodegenerative lysosomal storage disorders. The discovery that patients with homozygous PGRN mutations present with a lysosomal storage disorder suggests that the pathogenesis caused by progranulin deficiency could be dose-dependent and that PGRN mutations that lead to FTD may cause lysosomal dysfunction. Our current research has demonstrated that a decreased level of progranulin does significantly impair lysosomal proteolysis. We hypothesize that progranulin, or individual granulins, can alter the expression or activity of lysosomal enzymes. A preliminary screen to determine the effect of progranulin expression on lysosomal enzymes demonstrated that cathepsin D was particularly affected by progranulin expression. Furthermore, we demonstrated that progranulin interacts with cathepsin D and can increase its activity *in vitro*. Taken together, these experiments suggest that progranulin, or individual granulins, may act as an activator of cathepsin D. This progranulin-cathepsin D interaction may provide a potential mechanism by which progranulin haploinsufficiency leads to lysosomal dysfunction and contribute to our understanding of the pathogenesis that leads to neurodegeneration in FTD patients with PGRN mutations.

Disclosures: C.B. Valdez: None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 514.27/AA9

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: TDP-43 pathology in the hippocampus of centenarians

Authors: *T. IWASE[1], M. YOSHIDA[2], Y. HASHIZUME[3];
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Abstract: Transactivation response element DNA-binding protein 43 kDa (TDP-43) is a major component of the inclusions in frontotemporal lobar degeneration and sporadic amyotrophic lateral sclerosis. TDP-43-positive inclusions are also detected in cases of Alzheimer's disease, Lewy body disease, and argyrophilic grain disease. Concomitant mixed pathologies related to these diseases exist in aging brain, and the incidence of TDP-43 pathology in cognitively unimpaired subjects varies greatly (3% to 40%). Therefore age-related TDP-43 deposition is not clear. We previously studied TDP-43-positive inclusions in the hippocampus of centenarians using non-phospho specific TDP-43 antibody and found a high prevalence of TDP-43 positivity. In this study, we reevaluated incidence and extent of TDP-43-positive structures in centenarians using anti-phosphorylated TDP-43 antibody. The autopsied brains from 30 centenarians were studied. TDP-43-positive 6 cases in the previous study were included. In all cases, the pathologies of the most commonly seen in the aging brain were confirmed with Gallyas-Braak silver staining, and immunostainings for amyloid-β, phosphorylated tau (AT8) and α-synuclein. We performed phosphorylated TDP-43 immunostaining of the brain sections from the region including hippocampus, parahippocampal gyrus and adjacent temporal neocortex. We found TDP-43-positive neuronal cytoplasmic inclusions in 10 cases. Among the 10 cases, 4 cases were complicated with high Alzheimer's disease neuropathologic change. Lewy body pathology (limbic type) was found in 1 case. Argyrophilic grains were found in 3 cases (2 Saito stage II, 1 stage III). 2 cases had tangle-predominant dementia pathology. Only 1 case was solely with TDP-43-positive structures. TDP-43 depositions involved hippocampus and entorhinal cortex, but did not extend to the temporal neocortex. Our findings indicate a high prevalence of TDP-43-positive structures with limited distribution in centenarians. Age-related TDP-43 pathology in specific brain areas is supposed to exist, however, the assessment is difficult because of the co-existence of mixed pathologies associated with TDP-43 deposition in centenarians.

Disclosures: T. Iwase: None. **M. Yoshida:** None. **Y. Hashizume:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

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Program#/Poster#: 514.28/AA10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KNIH 2016-NG62003-00

Title: Discovery of potential autoantibody biomarkers for the detection of Alzheimer's disease

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Abstract: Autoantibody is known to be associated with autoimmune and neurodegenerative diseases as well as cancers. Anti-neuronal autoantibodies have been detected in neurodegenerative disease serum but their significance remains to be further investigated. In order to discover diagnostic biomarkers of Alzheimer's disease (AD), we analyzed serum autoantibody profiles from AD patients (n = 5) and cognitively normal control subjects (n = 5) using the HuProt human proteome microarray. The AD group exhibited more numbers of autoantibodies (98.0 ± 39.9 per person) than the normal control group (66.0 ± 39.6 per person). Among 434 autoantibodies detected in all subjects, 66.5% of autoantibodies (n=289) represented the occurrence number of one out of 10 subjects while 1.2% of autoantibodies (n=5) occurred in all 10 subjects. Functional analysis revealed that 47 AD-abundant autoantibodies were annotated to antigen proteins enriched in non-membrane-bounded organelle and cytoskeleton and ErbB signaling pathway. After validating proteome chip results using ELISA, five candidate AD-associated autoantibodies were subjected to subsequent ELISA-based validation in age- and sex-matched three different groups including AD (n=44), amnestic mild cognitive impairment (n=44), and cognitively normal subjects (n=44). In particular, the autoantibody XXX IgG levels were significantly higher in AD patients than cognitively normal control (p=0.02). Moreover, anti-XXX IgG levels were significantly correlated with mini-mental state examination (MMSE) scores (r_s=-0.204, p=0.019). The present study supports the presence of AD-associated autoantibodies can be used as potential biomarkers for diagnosis of AD.

Disclosures: **J. Jeon:** None. **S. Shim:** None. **S. Yun:** None. **J. Song:** None.

Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant ES19267

VDL fund

ISU Wildlife initiative

Title: Organotypic slice culture assay coupled to RT-QuIC (OSCAR) assay: towards rapid translational modeling of prion diseases

Authors: *N. KONDRU[1], S. MANNE, 50010[2], J. GREENLEE[4], M. GREENLEE[2], P. HALBUR[3], A. KANTHASAMY[2], A. KANTHASAMY[2];
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Abstract: Protein misfolding is a key pathological event in many neurodegenerative diseases like prion diseases, synucleinopathies and tauopathies that are collectively termed protein misfolding disorders (PMD). Prions serve as a prototypic model to study protein aggregation mechanisms and therapeutic development. Several attempts to develop anti-prion therapeutics have been impeded due to the lack of screening models that replicate a broad array of prion strains and the lack of a sensitive high-throughput biological system with reasonably short processing times. In addition to this, the differing outcomes of already tested compounds in different prion strains and species hindered the advancement of high-throughput screening. Therefore, a sensitive model encompassing prion replication and neurotoxicity would be indispensable to the pursuit of therapeutics for PMD. In this study, we present an ultrasensitive screening system coupled to an *ex-vivo* prion organotypic slice culture model to further accelerate progress towards rationale-based high-throughput therapeutic strategies. We developed a hybrid, organotypic slice culture assay coupled to RT-QuIC assay (OSCAR) that permits rapid, sensitive, specific and quantitative detection of prions from slice cultures, making it amenable to high-throughput screening. Additionally, the anti-prion activity of compounds can be resolved based on the power and kinetics of seeding activity. We used the OSCAR model to characterize the time-dependent augmentation of prion seeding activity. Furthermore, we determined that the prions generated in slice cultures can be efficiently passed to the second generation of slice cultures. Collectively, our results suggest that OSCAR is a robust model of prion diseases that offers increased versatility and a promising platform for understanding prion

proteinopathies as well as advancing anti-prion therapeutics (ES19267, ISU Wildlife Initiative, VDL fund).

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Poster

514. Alzheimer's Disease: Beyond Amyloid-Beta and Tau

Location: Halls B-H

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Program#/Poster#: 514.30/AA12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH, NINDS R01 grant 069566

Title: Post-translational modifications in the prion protein determine disease outcome

Authors: *P. AGUILAR CALVO[1], C. BETT[1], H. ERANA[2], K. SOLDAU[1], J. CASTILLA[2], P. R. NILSSON[3], W. SUREWICZ[4], C. J. SIGURDSON[1];
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Abstract: Prion diseases are caused by the misfolding of the cellular prion protein (PrP^{C}) into a pathogenic β-sheet rich isoform (PrP^{Sc}) that accumulates mainly in the brain. Prions can misfold into a wide variety of PrP^{Sc} conformations, known as strains, that correlate to diverse biochemical properties and disease phenotypes, including differences in incubation times, clinical signs, histopathological lesion profiles, and PrP^{Sc} deposition patterns in the brain within the same host genotype. PrP^{C} is post-translationally modified during biosynthesis by the addition of zero to two N-linked glycans and a glycophosphatidylinositol (GPI) anchor that may increase the conformational spectrum of PrP^{Sc}. Previous studies have shown that passage of GPI-anchored prion oligomers into mice expressing GPI-anchorless PrP^{C} switches the prion morphology from diffuse aggregates to dense, fibrillar plaques, yet it is unclear whether the original prion conformation is maintained within the anchorless PrP^{Sc} fibrils. Here we use mice expressing GPI-anchored or GPI-anchorless PrP^{C} 1) to investigate how post-translational modifications impact the aggregated protein structure and disease phenotype in mice infected with four prion strains and 2) to identify the properties of the original GPI-anchored prions that are maintained within the GPI-anchorless fibril. We found that the four GPI-anchorless prion strains seemed to converge into a similar strain, as mice showed equivalent incubation periods and morphologically-identical vasotropic fibrillar plaques in the brain. However, remarkable

biochemical differences reminiscent of their respective original GPI-anchored strains were also identified among anchorless prions. Upon passage of one anchorless strain into mice expressing GPI-anchored PrP^{C}, a novel strain emerged with unique structural and phenotypic properties, revealing that the GPI-anchorless state can be a source of novel PrP^{Sc} conformations. The remaining three strains emerged from the anchorless state nearly identical to their original anchored strains suggesting that only the quaternary structure had changed in the anchorless state. Together, our findings establish that variations in post-translational modifications that occur in vivo (i) explain one source for the emergence of new protein aggregate conformations, and (ii) may only affect the quaternary structure yet profoundly alter the pathogenesis of prion diseases.

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Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.01/AA13

Topic: C.01. Brain Wellness and Aging

Title: A structure-based approach to identifying novel modulators of the Nrf2 transcription pathway

Authors: *T. CARRENO VELAZQUEZ[1], P. BESWICK[2], B. WAHAB[2], J. ATACK[2]; [1]Life Sci., Sussex Drug Discovery Centre, Univ. of Sussex, Brighton, United Kingdom; [2]Sussex Drug Discovery Ctr., Univ. of Sussex, Brighton, United Kingdom

Abstract: Oxidative stress results when the antioxidant response is not sufficient to balance the production of reactive oxygen species (ROS). Fortunately, the cell contains antioxidant defence pathways that are essential for cell survival. However, dysfunction and up-regulation of the oxidative stress pathways have been implicated in the pathogenesis of many neurodegenerative diseases, including, for example, Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) protein is an important transcription factor that ordinarily is sequestered in an inactive form within the cytoplasm by association the Kelch-like erythroid cell-derived protein with CNC homology-associated protein (Keap1) protein. However, when it senses oxidative stress, Nrf2 dissociates from Keap1 and translocates to the nucleus to activate the transcription of antioxidant enzymes such as the phase II detoxifying enzymes glutathione S-transferase (GST), quinone reductase (QR), heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO1). Modulation of the Nrf2

pathway is therefore an attractive cellular pathway for drug discovery. In order to identify a novel Nrf2 activator that disrupts the Nrf2-Keap1 protein-protein interaction, we performed a virtual screen based upon the known structure of the protein interface and evaluated compounds in a DiscoveRx nuclear complementation assay that measures the translocation of Nrf2 from the cytoplasm to the nucleus. In this assay, sulforaphane was used as a positive control and had an EC_{50} of 400 ± 55 nM. In silico screening of a library of over 3 million compounds identified a number of virtual screening "hits" of which 122 were purchased and evaluated further. Of these compounds, five produced an activation of 15%, 20%, 25%, 40% and 100% relative to the sulforaphane control (which was defined as 100% activity). However, the use of a ROS-Glo assay showed that three of these five compounds were activating the pathway non-specifically by themselves producing free radicals rather than disrupting the protein-protein interaction. The remaining two compounds are currently being characterised further.

Disclosures: T. Carreno Velazquez: None. **P. Beswick:** None. **B. Wahab:** None. **J. Atack:** None.

Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.02/AA14

Topic: C.01. Brain Wellness and Aging

Support: Research support, Omniactive Health Technologies

Title: Effects of macular carotenoid supplementation on brain-derived neurotrophic factor and pro-inflammatory cytokines

Authors: *N. T. STRINGHAM[1], P. V. HOLMES[1], J. M. STRINGHAM[2];
[1]Neurosci., [2]Physiol. and Pharmacol., Univ. of Georgia, Athens, GA

Abstract: Purpose: Oxidative and inflammatory processes play a major role in stress-induced neural atrophy. There is a wide body of literature linking oxidative and inflammatory stress with a reduction in both neurotrophic factors and psychological stress resilience. We investigated the potential for the dietary carotenoids lutein, zeaxanthin, and mesozeaxanthin (the "macular carotenoids" [MC]), which have high antioxidant / anti-inflammatory capacity, to increase brain-derived neurotrophic factor (BDNF) and reduce concentrations of the pro-inflammatory cytokines TNF-α, IL-6, and IL-1β. Methods: 59 young (18-25 yrs.), healthy subjects participated in a 6-month, double-blind, placebo-controlled trial to evaluate the effects of MC supplementation serum BDNF, TNF-α, IL-6, and IL-1β. Serum antioxidant potential (AOP) and

the retinal concentration of MCs (termed macular pigment optical density [MPOD]), were also measured. Blood was obtained via fasting blood draws. All parameters were assessed at baseline and 6 months. Subjects were randomly assigned to one of three groups: placebo, 13 mg, or 27 mg / day total MCs. BDNF and the cytokines were assessed via ELISA, and MPOD via customized heterochromatic flicker photometry. Results: BDNF, MPOD, and AOP all increased significantly versus placebo in both treatment groups over the 6 month study period ($p < 0,05$ for all). By contrast, IL1-β decreased significantly versus placebo in both treatment groups ($p = 0.0036$ and $p = 0.006$, respectively). In terms of changes over the course of the study, significant relationships were determined for BDNF and IL1-β ($r = -0.47$; $p < 0.001$), and for BDNF and TNF-α ($r = -0.41$; $p = 0.0014$). No significant changes in any parameter were found for the placebo group. Conclusions: 6 months of daily supplementation with at least 13 mg of MCs significantly reduces serum IL1-β, and significantly increases serum BDNF, MPOD, and AOP. Although increases in AOP and MPOD were not directly related to decreases in IL-1β and BDNF, it is reasonable to suggest that the effects on IL-1β and BDNF are due to the increase in systemic and perhaps local (neural) antioxidant / anti-inflammatory capacity in our treatment groups. The significant relationships between the change in BDNF and both IL-1β and TNF-α over the course of the study suggest that regular consumption of MCs can interrupt the inflammatory pathway that leads to reduction of BDNF.

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Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.03/AA15

Topic: C.01. Brain Wellness and Aging

Support: NRF Grant 2011-0030049

Title: The effects of cellular senescence through the regulation of ubiquitin proteasome system in cholinergic neuron

Authors: *J. KIM, J. JANG, S. KANG, H. SEO;
Hanyang Univ., Ansan, Korea, Republic of

Abstract: Cellular senescence with progressive decline of its function implies the biological aging of an organism. Previous studies showed the dysfunction of protein degradation in aging process. In this study, we determined the effects of cellular senescence through the regulation of ubiquitin proteasome system in cholinergic neuron. We found that the chymotrypsin-like activity of ubiquitin proteasome system was down-regulated in several senescence conditions. However, ubiquitin-dependent proteasome activity was up-regulated in senescence conditions. When parkin, E3 ligase, over-expressed in cholinergic neuron, it increased the activities of ubiquitin proteasome system. We also detected the effects of parkin on cell viability and the expression of aging factors including hypoxia-inducible factor 1-alpha (HIF1-alpha). These results indicate that cellular senescence environment induces dysfunctional protein degradation and that the down-regulated cellular activities under senescence condition can be improved by the regulation of ubiquitin proteasome system, suggesting potential future therapeutic approach for senescence related diseases.

Disclosures: J. Kim: None. **J. Jang:** None. **S. Kang:** None. **H. Seo:** None.

Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.04/AA16

Topic: C.01. Brain Wellness and Aging

Title: Telomere length dynamics and telomerase activity in the aging murine brain

Authors: Q. AIN[1], A. KRETZ[1], D. PENNDORF[1], M. FISCHER[2], T. BONDEVA[3], O. WITTE[1], *C. W. SCHMEER[1];
[1]Hans Berger Dept. of Neurol., Jena, Germany; [2]Dept. of Intrnl. Med. II, Jena, Germany; [3]Dept. of Intrnl. Med. III, Jena, Germany

Abstract: Telomeres are repetitive nucleotide sequences that maintain chromosome stability and cell replicative capacity. Critical telomere shortening by repeated cell division triggers cellular senescence in replicative tissue and organs, and is considered a strong paradigm to explain aging in general. However, a contribution of telomere dysfunction, or shortening to the mechanism of physiological brain aging is still uninvestigated. The post-replicative state of the mature brain appears to be nominal, since neurons can re-induce unscheduled cell cycle (CC) activity under stress conditions, and glia populations preserve a life-long replicative capability. Therefore, in

this study, we addressed telomere length dynamics in the murine brain during physiological aging and determined the effect of increasing life-time on the catalytic activity of TERT, an enzyme which elongates telomeres in DNA strands, and directly influences age-related gene transcription, e.g. by interaction with NF-κB. Relative telomere length (RTL) as a function of age was assessed in cortical neural cells by Flow-FISH in a CC-dependent manner. TERT activity was analyzed in cortical tissue extracts by a qPCR-based TRAP assay. Levels of NF-κB expression were determined by real time qPCR in corresponding tissue.
Results from our study show that RTL remains stable in cells belonging to G_0/G_1 phase of the CC, which are most likely neurons. In contrast, we detected a significant age-dependent reduction in RTL by ~54% for a portion of brain cell populations in G_2 phase. Moreover, we found an increase in the S/G_2 phase population at the expense of the G_0/G_1 fraction, indicating a shift in CC activity. The identity of these cells still has to be specified, however, is likely to include neurons with instable DNA or CC reactivation, and dividing glia populations. TERT activity remained stable upon increasing age, which might point to alternative telomerase functions within the mature brain. In support of this and the feed-forward interaction between TERT and the RelA subunit of NF-κB, we found a significant age-related up-regulation of RelA mRNA, whereas c-Rel expression was down-regulated. In summary, our data provide evidence for unexpected age-related telomere dynamics in the postmitotic environment of the brain. Considering CC- and telomere-related pathomechanisms might contribute to a better understanding of physiological brain aging and age-related CNS disorders.

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Poster

515. Oxidative Stress in Neurodegeneration

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Program#/Poster#: 515.05/AA17

Topic: C.01. Brain Wellness and Aging

Support: NRF Grant 2012R1A6A1028677

Title: The role of brain-derived neurotrophic factor in Capsosiphon fulvescens proteins-induced cognitive enhancement

Authors: *J. OH;
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Abstract: Reactive oxygen species (ROS) causes functional declines in many organs of the body including the brain, and antioxidants can reduce these ROS-induced neuronal damage with age. Extracts form *Capsosiphon fulvescens* (*C. fulvescens*) contain various antioxidants including protein, polysaccharide, etc. In this study, effects of crude proteins of *C. fulvescens* (Cf-CPs) on brain-derived neurotrophic factor (BDNF) and calcium-dependent protein kinases were investigated. Following extraction with 0.1 M sodium acetate (pH 6) and methanol precipitation, Cf-CPs were freeze-dried and saline soluble proteins were used. Rats were given 14 daily oral administration of saline or Cf-CPs (100 and 200 mg//kg) and then sacrificed at different time points (30 and 90 min) after the final administration. Oral administration of Cf-CPs significantly upregulated expression of mature and truncated BDNF in the rat hippocampus at 90 min post administration. At the same time point, phosphorylation of calcium/calmodulin-dependent protein kinase II was significantly increased in a dose-dependent manner. These finding suggest that Cf-CPs regulate activation of calcium-dependent protein kinase associated with BDNF in the rat hippocampus and could play an important role in learning and memory with age.

Disclosures: J. Oh: None.

Poster

515. Oxidative Stress in Neurodegeneration

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: C.01. Brain Wellness and Aging

Support: Supported by Beaumont Health's Neuroscience Center of Excellence and a generous donation from Ms. Marilyn Bishop

Title: Antioxidant regulator nrf2 increases with age, while molecular chaperone HSPA8 decreases, in CSF from healthy subjects: associations with oxidative stress

Authors: *D. A. LOEFFLER[1], A. C. KLAVER[2], M. P. COFFEY[1], J. O. AASLY[3], P. A. LEWITT[4,5];
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Abstract: Age-associated declines in antioxidant defense and protein homeostasis mechanisms ("proteostasis") are thought to contribute to age-related neurodegenerative disorders. Decreased brain antioxidant activity results in oxidative stress, which can activate a key proteostasis process, chaperone-mediated autophagy (CMA). The literature contains conflicting reports for age-related changes in experimental animals for nuclear factor erythroid 2-related factor 2 (nrf2),

a “master regulator” of the antioxidant response, and heat shock 70-kDa protein 8 (HSPA8), a molecular chaperone involved in multiple proteostasis mechanisms including CMA. The objective of this study was to explore age-related changes in these proteins, and their associations with oxidative stress and anti-oxidant biomarkers, in human cerebrospinal fluid (CSF). We examined correlations between age and CSF nrf2, HSPA8, 8-hydroxy-2’-deoxyguanosine (8-OHdG), 8-isoprostane (8-ISO), and total antioxidant capacity (TAC) in CSF samples from 34 healthy subjects ages 20-75. Age was positively associated with nrf2 (Spearman rho = 0.47; p = .005) and negatively associated with HSPA8 (rho = -0.47; p = .005). An age-related increase in oxidative stress was suggested by a positive association between age and 8-OHdG (rho = 0.61; p = .0001). 8-OHdG was moderately positively associated with nrf2 (rho = 0.32; p = .06) and strongly negatively associated with HSPA8 (rho = -0.58; p = .0004). These findings suggest that during healthy aging, CSF levels of nrf2 may increase while HSPA8 levels may decrease. An age-related increase in oxidative stress may contribute to these alterations. Further studies are indicated to determine if the age-related changes in CSF nrf2 and HSPA8 reflect similar changes in the brain.

Disclosures: **D.A. Loeffler:** None. **A.C. Klaver:** None. **M.P. Coffey:** None. **J.O. Aasly:** None. **P.A. LeWItt:** F. Consulting Fees (e.g., advisory boards); consultant or advisor for Acorda, Biogen Idec, Britannia, Insightec, Intec, Kyowa Hakko, Lundbeck, Luye, NeuroDerm, Pfizer, ProStrakan, Prothena, SynAgile, Teva, UCB Pharma, and USWorldMeds.. Other; clinical research grant support from AbbVie, Acorda, Adamas, Biotie, Kyowa Hakko, The Michael J. Fox Foundation for Parkinson’s Research, the Parkinson Study Group, Pharma 2B, and USWorldMeds.

Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.07/BB1

Topic: C.01. Brain Wellness and Aging

Title: Resveratrol effect on the Sirtuins regulation during aging process

Authors: M. F. FERRO-ABELLA[1], R. RAMIREZ-Y AYALA[1], J. C. MORALES[2], E. M. BRAMBILA[1], G. FLORES[3], L. M. PEREZ-PEÑA[4], *P. AGUILAR-ALONSO[5]; [1]BENEMERITA UNIVERSIDAD AUTONOMA DE PUEBLA, PUEBLA, Mexico; [2]Ctr. de Investigación en Reproducción Animal. CINVESTAV-IPN. UAT, TLAXCALA, Mexico; [3]Lab. de Neuropsiquiatría, Inst. de Fisiología, BUAP, PUEBLA, Mexico; [4]CENTRO DE QUIMICA, BUAP, PUEBLA, Mexico; [5]BUAP, Puebla, Mexico

Abstract: The aging process is a biological phenomenon that concerns all population sectors, the individual productive life decrease and his physical, emotional and cognitive functions are affected by aging. It has been noted that the uptake of resveratrol mimics some caloric restriction characteristics, process in which un member of Sirtuin family, the SIRT1 protein, is involved. In the aging rat experimental model, it is still unclear the effect that the chronic administration of resveratrol has on the SIRT family member's expression. The objective of this study was to assess the effect of resveratrol chronic administration (10 mg/kg) on cognitive performance and to clarify any change of the level of expression in the members of SIRT family 7 members level expression. On this Long-term evaluation, resveratrol shows an important decrease in nitrites and lipid peroxidation products' production showing its antioxidant property. It was observed that the cytoarchitecture of the regions CA1 and CA2 region in hippocampus in rats treated with resveratrol keep their integrity with the passing pf time and the behavioral test shows cognitive performance improvements on rats with chronic administration of resveratrol during 8 months. Regarding the transcription regulation. The administration of resveratrol for 2 months promotes changes in the regulation of SIRT1 and after 8 months of vitamin E administration the regulation of SIRT3 is positive. Nevertheless, significant changes weren´t found in other Sirtuin member during the resveratrol administration. This shows that the chronic administration of resveratrol is a determining factor in the reduction of oxidative stress and conservation of the hippocampus integrity in rats, as well as their cognitive performance.

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Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.08/BB2

Topic: C.01. Brain Wellness and Aging

Support: AG047612

Title: Neuromuscular excitability and synaptic transmission in *Drosophila* Superoxide Dismutase (Sod) mutant larvae

Authors: ***A. UEDA**, C.-F. WU;
Univ. of Iowa, Iowa City, IA

Abstract: It has been established that reactive oxygen species (ROS) modulate the function of a variety of ion channels. However, identifying and analyzing the consequences of ion channel

redox regulation in membrane excitability and synaptic transmission await further exploration. The Sod gene in Drosophila encodes the cytoplasmic Cu/Zn superoxide dismutase (Cu/Zn Sod), which is an essential component in a major antioxidant defense pathway for scavenging ROS generated during aerobic respiration and other metabolic processes. Drosophila Sod mutant flies show increased sensitivity to ROS generating agents, such as paraquat, and greatly decreased lifespan. We found that defects in both axonal excitability and synaptic transmission could be detected in the larval neuromuscular preparation of Sod mutants. Upon high frequency nerve stimulation, Sod motor axons displayed supernumerary action potentials, resulting in greatly enhanced synaptic transmission, composed of superimposing excitatory junctional potentials (EJPs). Furthermore, spontaneous miniature EJPs (mEJPs) in Sod larvae were significantly smaller compared to WT, even though nerve-evoked EJPs appear normal, indicating that the number of synaptic vesicles released upon arrival of individual nerve action potentials can be increased by Sod mutations. This suggests an increased quantal content, a potential homeostatic mechanism for sustaining transmission efficacy (EJP size maintenance). Our results demonstrated that defects in *Sod* enzymatic function can cause nerve excitability and synaptic transmission defects even at an early developmental stage, even though the Sod mutant fly can undergo pupation and its lethality phase occurs in adulthood. This finding suggests a possibility of defining early predictive physiological indicators for late-onset Sod-related neurodegenerative disorders.

Disclosures: **A. Ueda:** None. **C. Wu:** None.

Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.09/BB3

Topic: C.01. Brain Wellness and Aging

Title: Nature-inspired new hybrids to counteract oxidative stress in neurodegeneration

Authors: M. M. SERAFINI[1], L. POLONI[1], M. RONFANI[1], M. GALASSO[1], *M. RACCHI[2], M. BARTOLINI[3], M. ROSINI[3], C. LANNI[1];
[1]Dept. of Drug Sci. - Univ. of Pavia, Pavia, Italy; [2]Univ. Pavia, Pavia 27100, Italy; [3]Dept Pharm. and Biotech. - Univ. of Bologna, Bologna, Italy

Abstract: Background: Oxidative stress is a common feature of different neurodegenerative disorders. The identification of new drugs acting on oxidative stress pathways is an open field of research because of their potential application in different pathologies. Natural products offer great chemical diversity and have already proven to be a rich source of therapeutics. Polyphenols

are widely diffused in nature. They have been shown to modulate several pathways, including oxidative injuries. Diallyl sulfides are garlic-derived organosulfur compounds carrying allyl mercaptan moieties. They counteract oxidative stress through antioxidant phase II enzymes expression. In our previous paper, we combined these molecular fragments in new chemical entities to produce hybrids with Aβ anti-aggregant and anti-oxidant activity (Simoni et al. 2015) which have been the basis for the synthesis of different new derivatives. These compounds have been tested in human neuroblastoma SH-SY5Y cells for their ability to counteract oxidative stress and to exert a neuroprotective effect.
Objective: In order to investigate the structure-activity relationship (SAR) our goal has been to test the anti-oxidant ability of the new derivatives and to investigate the pathways involved in this activity.
Methodology: To determine the potential interest of new nature-inspired molecules as antioxidants, we investigated their protective effects against H_2O_2-induced oxidative damage. The scavenging effect on reactive oxygen species (ROS) was evaluated in human neuroblastoma SH-SY5Y cells by using the fluorescent probe dichlorofluorescein diacetate (DCFH-DA) as a specific marker for the quantitative intracellular formation of ROS. Furthermore, the expression of proteins involved in the anti-oxidant phase II response has been investigated by RT-PCR and western blot.
Conclusions: The new nature-inspired molecules protect against H_2O_2-induced oxidative damage with different potencies, as assessed by DCFH-DA experiments. The most promising compounds were also tested for their mechanism of action, demonstrating their involvement in the anti-oxidant phase II response. In particular, a modulation of Nrf-2 and its targets has been observed.
Bibliography: Simoni E, et al. ChemMedChem. 2015

Disclosures: **M.M. Serafini:** None. **L. Poloni:** None. **M. Ronfani:** None. **M. Galasso:** None. **M. Racchi:** None. **M. Bartolini:** None. **M. Rosini:** None. **C. Lanni:** None.

Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.10/BB4

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant AG020251

NIH Grant AG037868

NIH Grant AG034605

Title: Sexual Dimorphism of the Hippocampal Transcriptome

Authors: ***N. M. PORTER**[1], L. D. BREWER[1], M. RAUHUT[2], K.-C. CHEN[1], O. THIBAULT[1], P. W. LANDFIELD[1], E. M. BLALOCK[1];
[1]Pharmacol. and Nutritional Sci., Univ. of Kentucky, Lexington, KY; [2]Dickinson Col., Carlisle, PA

Abstract: Many traits in mammals are sexually dimorphic including the sensitivity of brain cells to neurotransmitters or noxious stimuli/toxins. Further, sexual dimorphism is recognized to contribute to age-related differences in cognitive function, neurodegenerative disease pathology, disease outcomes and responses to therapy or drug treatment. However, very little is known regarding the underlying genomic basis of the sexual dimorphism of the brain. Here we investigated the difference in the hippocampal transcriptome of male and female rats to identify genes and genomic networks that may contribute to the dimorphism of hippocampal-dependent processes such as learning and memory. Twelve-month old, middle-aged male or female F344 rats were gonadectomized in order to study the sexual dimorphism of the transcriptome in isolation from the actions of circulating sex hormones. Other similar groups of gonadectomized male and female rats were treated with low, physiological levels of estradiol for six weeks to separately assess the action of this hormone on gene expression. Affymetrix microarrays were used to identify hippocampal genes that differed significantly between male and female rats. Those genes were then subjected to a DAVID analysis to identify the major categories and gene pathways that differed by sex. Our analyses revealed that 183 (of ~2000 tested) genes differed significantly by sex with a false discovery rate of 0.3. Of these 183 genes, 102 exhibited significantly higher expression in females compared to males. Among the genes with higher expression in females were genes that play a role in lipid and cholesterol metabolism and vascular function. In addition, several neuromodulators, neurotransmitter receptors and synaptic proteins important in plasticity were identified. Surprisingly, very few genes were altered in male or female rats by treatment with estradiol. Taken together, it appears possible that dimorphic gene expression may account for important sex differences in hippocampal-dependent functions.

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Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.11/BB5

Topic: C.01. Brain Wellness and Aging

Support: Korea Health Industry Development Institute(HI14C1943)

Title: The enhanced oxidative damage of sequestosome/p62 promoter in type 2 diabetic rats with age

Authors: *S. AHN, J. JEONG, J. IM, S. PARK;
Dept. of Neurol., Soonchunhyang Univ. Bucheon Hosp., Bucheon, Korea, Republic of

Abstract: Background - Sequestosome/p62 (p62) is frequently detected in the tau inclusions in Alzheimer disease (AD). We reported that the mRNA and protein levels of p62 decreased robustly with age in Otsuka Long-Evans Tokushima Fatty (OLETF) rats. However, the underlying mechanisms remain unknown. The objective of this study is to examine the association of oxidative damage of p62 promoter with the decrease of p62 transcription.
Materials and Methods - Diabetic OLETF rat and their corresponding non-diabetic controls, Long-Evans Tokushima Otsuka rats were utilized in this study. Rats were sacrificed at 32 or 60 weeks. Genomic DNA was isolated from brain tissue and purified. The formamidopyrimidine glycosylase (fpg) cleavage reaction was performed using genomic DNA. With the specific primers of p62 promoter real time polymerase chain reaction was employed. Then the levels of damaged DNA within the rat p62 promoter were quantified.
Results - The extent of oxidative damage to the p62 promoter containing a CpG island (>60%) was found increased in the brain of OLETF rats. Further, the oxidative modification was enhanced in an age-dependent manner.
Conclusions - These findings suggest that oxidative damage to the p62 promoter is the underlying mechanism of the decreased expression of p62 in diabetic rats. And the age-dependent increased oxidative damage may contribute to the age-associated neurodegenerative diseases in the model.
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Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.12/BB6

Topic: C.01. Brain Wellness and Aging

Support: BBSRC studentship

ISPG funding

Title: Identifying regulators of synaptic stability during normal healthy ageing in non human primates

Authors: *L. C. GRAHAM[1], I. S. AMORIM[1], T. H. GILLINGWATER[1], M. J. NALDRETT[2], S. G. KOHAMA[3], G. PENNETTA[1], P. A. SKEHEL[1], H. F. URBANSKI[3], T. M. WISHART[1]; [1]Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Donald Danforth Plant Sci. Ctr., St. Louis, MO; [3]Oregon Natl. Primate Res. Ctr., Beaverton, OR

Abstract: Humans, like many other mammals, are susceptible to age-related cognitive decline in the absence of neurodegenerative disease[1, 2, 3, 4]. The hippocampus and its complex projections appear to be the most vulnerable during normal healthy ageing[1, 2, 3] whereas other brain regions, such as the occipital cortex, are almost completely spared[4, 5, 6, 7]. In what appears to be the first study utilizing label-free proteomics on synaptic isolates from the non-human primate (NHP) brain, we have tracked regional synaptic protein expression profiles in the rhesus macaque during normal healthy ageing. Synaptosomes from 'spared' (occipital cortex) and 'vulnerable' (hippocampus) brain regions identified differential regulation (>20%) of over 200 proteins throughout the ageing timecourse. Further examination of these candidates *in silico* highlighted mitochondrial function and calcium binding differ between the 'spared' and 'vulnerable' regions - processes which have been widely described as contributors to neuronal vulnerability. By applying stringent filtering parameters to the proteomic and *in silico* data, we have identified several novel regulators of synaptic stability which have the propensity to promote morphological alterations *in vivo*. Experimental manipulation of such candidates at the Drosophila larval neuromuscular junction demonstrates robust phenotypes ($p<0.0001$). The data described illustrate how modern bottom-up proteomic methodologies may be utilized to identify potential candidates capable of modulating regional synaptic and neuronal stability. This study may be more relevant than equivalent rodent based studies for the identification of biomarkers in human ageing due to our unique phylogenetic relationship with NHP species.

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Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.13/BB7

Topic: C.01. Brain Wellness and Aging

Support: Alzheimer's Association Grant NPSPAD 247219

Title: Peptide Hormone amylin Reduces oxidative stress through improved mitochondrial dynamics

Authors: *A. S. PALIOBEIS[1], J. GRIZZANTI[2], S. PATRICK[2], G. CASADESUS[3,2];
[1]Biol. Sci., [2]Sch. of Biomed. Sci., [3]Dept. of Biol. Sci., Kent State Univ., Kent, OH

Abstract: Oxidative stress has been shown to be an early predictor and key pathological feature of neurodegenerative diseases including Alzheimer's disease (AD). Previous work in our laboratory has demonstrated that administration of the peptide hormone amylin can reduce levels of oxidative stress in a transgenic mouse model of AD (APP/PS1) as well as Neuroscreen-1 cultures (neuronal cell model). Although it is apparent that amylin has antioxidant activity, its mechanism of action remains unclear. Because mitochondria are the main producers of reactive oxygen species (ROS) that account for oxidative stress in a cell, they have been a target of our investigation. Specifically, we determined whether amylin treatment regulates mitochondrial associated proteins and mobility deficits in the APP/PS1 AD mouse model. Our data show that amylin treatment regulates mitochondrial dynamics-associated proteins as well as proteins associated with mitochondrial biogenesis. Taken together, our data suggest that some of the effects of amylin on oxidative stress regulation may stem from improving mitochondrial function.

Disclosures: A.S. Paliobeis: None. **J. Grizzanti:** None. **S. Patrick:** None. **G. Casadesus:** None.

Poster

515. Oxidative Stress in Neurodegeneration

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Program#/Poster#: 515.14/BB8

Topic: C.01. Brain Wellness and Aging

Support: Indian Council of Medical Research

International Brain Research Organisation

International Society for Neurochemistry

Title: WNIN/Ob obese rat as an appropriate animal model to study the neurobiology of premature aging

Authors: *S. GHOSH, J. K. SINHA, M. RAGHUNATH;
NATIONAL INSTITUTE OF NUTRITION, HYDERABAD, India

Abstract: Purpose: Proportion of aged individuals is on the rise globally and so is the burden of non-communicable diseases like obesity. Wistar of National Institute of Nutrition obese (WNIN/Ob) rat is a novel strain developed at NIN, Hyderabad, India. These rats have significantly reduced average lifespan of 15-18 months in contrast to 36 months in normal WNIN rats. Elucidation of various molecular and biochemical characteristics in these rats would help to establish it as an appropriate model to study the neurobiology of ageing and obesity. Methods: Different growth characteristics were studied and the lifespan analysis was performed using OASIS software. The neuronal and glial changes were studied using Nissl staining and immunohistochemistry. Levels of oxidative stress, antioxidant enzyme activity and extent DNA damage were studied in various brain parts. Results: The brain weights were significantly decreased and there was a 60% decrease in the total lifespan in the WNIN/Ob obese rats as compared to the lean littermates as well as WNIN normal rats. Various neuronal and glial changes were observed that are seen in the ageing brain. In addition, oxidative stress levels and extent of DNA damage were observed to be significantly high in the brain of young WNIN/Ob obese rats as compared to age-matched rats and it was as high as comparable to what observed in 15 months old WNIN normal rats. The levels of antioxidants enzyme activity were also significantly low in the WNIN/Ob obese rats. Conclusion: Onset of various degenerative features like increased oxidative stress, astrogliosis, DNA damage and decreased antioxidant levels in different brain regions of WNIN/Ob obese rats at a much younger age is a plausible cause of reduced longevity observed in this novel obese rat model. This model may be used to study the connecting link between obesity and ageing.

Disclosures: **S. Ghosh:** None. **J.K. Sinha:** None. **M. Raghunath:** None.

Poster

515. Oxidative Stress in Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 515.15/BB9

Topic: C.01. Brain Wellness and Aging

Support: R00AG037716

Title: Cyclophilin D associated brain mitochondrial F1FO ATP synthase dysfunction in aging mice

Authors: *E. GAUBA, L. GUO, H. DU;
Dept. of Mol. & Cell Biol., Univ. of Texas At Dallas, Richardson, TX

Abstract: Mitochondrial dysfunction is a pronounced brain pathology closely associated with declined cognitive function accompanying brain aging. Among the recognized aging-related mitochondrial abnormalities, F1FO ATP synthase dysfunction has been proposed to be a critical mitochondrial defect. However, the molecular mechanisms causing the dysfunction of this key mitochondrial enzyme in aging brains still remains unresolved. Cyclophilin D (CypD), a mitochondrial peptidylprolyl isomerase is the key regulator of mitochondrial permeability transition pore (mPTP). Recent studies suggest that F1FO ATP synthase subunit Oligomycin Sensitivity Conferring Protein (OSCP) is a binding partner of CypD. The interaction of CypD with OSCP affects the integrity of F1FO ATP synthase, thus leading to lowered oxidative phosphorylation (OXPHOS) efficiency and sensitized mPTP formation. In view of our previous findings of increased CypD expression in aging animals as well as in aging human subjects, the objective of this study is to determine whether CypD modulates F1FO ATP synthase deregulation in aging brain. By immunoblotting we have found that increased CypD expression, enhanced CypD/OSCP interaction and selective loss of OSCP are prominent brain mitochondrial changes in aging mice. Additionally, further mitochondrial functional assays showed that brain mitochondria from the aging mice demonstrated decreased F1FO ATP synthase catalytic activity and defected F1FO complex coupling, along with lowered OXPHOS capacity and sensitized mPTP formation. To determine the impacts of CypD, we employed CypD deficient mice and found such aging-related F1FO ATP synthase deregulation was largely mitigated by the depletion of CypD. Therefore, the simplest interpretation of this study is that CypD promotes F1FO ATP synthase dysfunction and the resultant mitochondrial deficits in aging brains. Increased CypD expression, enhanced CypD/ OSCP interaction and loss of OSCP may constitute primary mitochondrial defects in aging brains; and the aging-related brain mitochondrial F1FO ATP synthase dysfunction could be at least partly protected by the inhibition of CypD.

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Poster

515. Oxidative Stress in Neurodegeneration

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Topic: C.01. Brain Wellness and Aging

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Title: Toward a comprehensive metallome of the marmoset brain: manganese, iron, copper, and zinc distribution in the cerebral cortex

Authors: *B. KNAUER[1,3], D. J. HARE[4,5], B. PAUL[6], P. MAJKA[2,7,8], M. G. P. ROSA[1,8], D. H. RESER[2];
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Abstract: Objective: Biometals are essential for normal brain function and metal dysregulation is implicated in a number of disease states. However, the distribution of metal ions in normal brain tissue has not been well characterized in primates. Here we employed laser ablation – inductively coupled plasma – mass spectrometry (LA-ICP-MS) to map the tissue distribution of manganese (^{55}Mn), iron (^{56}Fe), copper (^{63}Cu), and zinc (^{66}Zn), across the cerebral cortex of the adult common marmoset (*Callithrix jacchus*) at multiple spatial resolutions.
Methods: Brain tissue from 4 adult marmosets was obtained from other experiments in our laboratory or from archived specimens (preserved in 4% PFA, cryoprotected, and used fresh or frozen at -80º C). In one case, the cortical hemisphere caudal to the lateral sulcus was flat mounted, including V1 and most extrastriate visual areas. Remaining cerebral hemispheres were sectioned at 40 µm in the coronal or sagittal planes and mounted uncoverslippped on glass slides for LA-ICP-MS (laser spot size of 50, 60, or 100 µm). Adjacent series were stained for histological markers including Nissl, myelin, cytochrome oxidase (CO), and calbindin. LA-ICP-MS output was aligned to histological data, with special focus on the primary visual, auditory,

and somatosensory cortical areas. Australian NHMRC guidelines encourage the use of scavenged tissue for research, and all specimens were obtained from studies approved by the Monash Animal Research Ethics Committee.
Results: In comparison to white matter, grey matter showed higher concentrations for all the metal ions investigated. In coronal and sagittal sections the change in ^{55}Mn and ^{56}Fe concentrations demarcated known anatomical boundaries of the primary sensory areas. Regions with dense CO staining were associated with high ^{55}Mn and ^{56}Fe concentrations. In the flat mounted preparation, high ^{55}Mn and ^{56}Fe concentrations were evident in early visual areas, but individual CO blobs or stripes could not be resolved.
Conclusions: The marmoset brain is an ideal target for mapping the complete distribution of redox metals, as the small, yet highly differentiated brain allows for identification of regions of interest for further study of traumatic and age-related changes in metal concentrations.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.01/BB11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: The role of IL-1 signaling in behavior and neurodegeneration after soman-induced convulsions

Authors: *T. M. FERRARA-BOWENS, J. K. CHANDLER, J. F. IRWIN, K. LAITIPAYA, D. D. PALMER, E. A. JOHNSON;
Pharmacol., USAMRICD, Gunpowder, MD

Abstract: Chemical warfare nerve agent (CWNA) exposure initiates convulsive activity resulting in status epilepticus (SE), which leads to severe neuropathology and behavioral impairment in rodents. While treatments are available to ameliorate SE, the therapeutic window for control is relatively short, with no approved treatments available to address the neurodegenerative process. Following exposure, brain damage resulting from SE activates microglia and astrocytes to upregulate pro-inflammatory cytokines including IL-1α, IL-1β, IL-6, and TNFα. This creates a positive feedback loop to exacerbate neurodegeneration and to damage potentially healthy neuronal tissue. A soman (GD) model was developed using wild-type and IL-1 signaling knockout (KO) mouse strains (i.e., IL-1R1 and IL-1Ra) to validate IL-1 signaling as

a viable neuroprotective target. Behavioral studies were conducted with the Open Field, Zero Maze, and Barnes Maze to assess mobility, anxiety, and cognitive function. In a separate study, anakinra, the human recombinant IL-1Ra, was used to treat GD-exposed WT mice. Results showed that the absence of IL-1R1 improves neuropathology over time and attenuates anxious behavior, whereas the absence of IL-1Ra worsens brain damage and does not improve anxiety. Hyperactivity does not improve with the absence of IL-1R1 or IL-1Ra, nor does it prevent spatial learning and memory, but the absence of IL-1Ra worsens damage in the CA2/3 region of the hippocampus, which may account for the slowed learning and memory in these mice over time. Finally, anakinra is neuroprotective 24 hours after convulsion onset. These results show that therapeutically targeting the IL-1 signaling pathway is necessary to attenuate brain damage, and therefore, research into neuroprotective strategies is important to improve brain pathology and potentially improve behavioral outcomes. The views expressed in this talk are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. This research was funded by DTRA. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KAKENHI25460098

KAKENHI24390016

Title: Pathophysiological role of TRPM2 in a mouse model of chronic cerebral hypoperfusion

Authors: J. MIYANOHARA[1], *H. SHIRAKAWA[1], K. NAGAYASU[1], T. NAKAGAWA[1,2], S. KANEKO[1];
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Abstract: Vascular dementia (VaD) is one of the most common forms of cognitive disorder, responsible for more than 20% of cases of dementia. It has been recognized that cognitive impairment in VaD is highly associated with inflammation in both animal and human, suggesting that regulation of inflammatory response is potential target for cognitive decline in VaD. Transient receptor potential melastatin 2 (TRPM2), a Ca^{2+}-permeable nonselective cation channel, is functionally expressed in the brain and immune cells, implying that TRPM2 could be involved in inflammation during chronic cerebral hypoperfusion. In this study, wild-type (WT) and TRPM2-knockout (KO) mice were subjected to bilateral common carotid artery stenosis (BCAS) using microcoil with 0.18 mm diameter. At 28 days after BCAS, cognitive impairment and white matter injury were significantly attenuated in TRPM2-KO mice compared with WT mice. Immunohistochemical analysis revealed that the accumulation of Iba1-positive microglia/macrophages in corpus callosum was reduced in TRPM2-KO mice, whereas no change was observed in the number of GFAP-positive astrocytes and Gr1-positive neutrophils. In addition, we found that accumulation of microglia/macrophages in corpus callosum area and the cognitive impairment observed in vehicle-treated BCAS mice were reduced in mice treated with minocycline, an inhibitor of microglia/macrophage activation at a dose of 50 mg/kg per day for 28 days after the BCAS surgery. These results indicate that TRPM2 is involved in chronic cerebral hypoperfusion-induced cognitive impairment and white matter injury by regulating microglia/macrophages activation.

Disclosures: J. Miyanohara: None. **H. Shirakawa:** None. **K. Nagayasu:** None. **T. Nakagawa:** None. **S. Kaneko:** None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.03/BB13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Korea Healthcare Technology R&D Project, Ministry of Health & Welfare HI15C1928

Title: Upregulation of microglial toll-like receptor 4 induced by prothrombin kringle-2 contributes to neurotoxicity in the hippocampus *In vivo*

Authors: S. KIM[1,2], H. JANG[1,2], U. JUNG[5], *S. KIM[1,2,3,4];
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Abstract: We have recently reported that overexpression of prothrombin kringle-2 (pKr-2), which is a domain of prothrombin that is generated by active thrombin, induces microglial activation in the substantia nigra (SN) of mouse brains, resulting in neurodegeneration in the nigrostriatal dopaminergic system. In addition, we found that patients with Parkinson's disease (PD) showed a significant increase in pKr-2 expression in the SN compared with age-matched controls, suggesting that there might be a correlation between pKr-2 expression and parkinsonism in patients. However, it is still unknown whether there is any correlation between pKr-2 and neurotoxicity in the hippocampus of adult brains, even though there was a report showing that the level of active thrombin was significantly increased in the hippocampus of patients with Alzheimer's disease (AD), suggesting that pKr-2 might be also increased in the lesioned hippocampus. Here, we report that the levels of pKr-2 and toll-like receptor 4 (TLR4) are upregulated in the hippocampus of patients with AD, and intra-hippocampal injection of pKr-2 induces a significant increase in microglial TLR4 expression, resulting in microglial activation and neurotoxicity in the hippocampus of mouse brains. Moreover, pKr-2-induced neurotoxic events were significantly diminished in TLR4-deficient mice compared with normal mice. Therefore, our results suggest that pKr-2 may be a pathogenic factor in neurodegenerative diseases such as PD and AD, and that the inhibition of pKr-2-induced microglial TLR4 may be protective against neurodegeneration in the adult brains. **Acknowledgements:** This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (2014R1A1A2056508 and 2014R1A1A4A01007858), and also by grants from the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare (HI15C1928).

Disclosures: S. Kim: None. **H. Jang:** None. **U. Jung:** None. **S. Kim:** None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

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Program#/Poster#: 516.04/BB14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Swedish Research Council

Swedish Alzheimer Fundation

Spanish Research Council

Multipark

Title: Lack of Galectin-3 alters microglial phenotype and affects Alzheimer's Disease Progression in 5xFAD the mouse model .

Authors: *A. BOZA-SERRANO;
Lund Univ., Lund, Sweden

Abstract: Background: Alzheimer's Disease (AD) is the most prevalent type of dementia. The two main pathological hallmarks most commonly used to describe the pathology are the extracellular protein aggregates of amyloid beta (Aβ), called senile plaques, and the formation of intracellular neurofibrillary tangles. Previous studies have shown that Aβ can activate microglial cells, inducing an inflammatory response believed to contribute to the neurodegenerative process in AD. Galectin-3 (gal3) is involved in microglial activation and inflammation. Therefore, we have investigated whether gal3 if the lack of gal3 is able to protect against disease progression in the 5xFAD mouse model of AD.
Results: Challenging microglial cells with Aβ induced a significant increase in the levels of pro-inflammatory mediators including the inducible Nitric Oxide Synthase (iNOS) and pro-inflammatory cytokines. Reducing the Gal3 levels or activity led us to a significant reduction of the observed inflammatory response induced by Aβ fibrils. Using an AD mouse model (5xFAD) we studied if the lack of gal3 is able to reduce the pathology *in vivo*. A significant reduction in Aβ40 was observed at 6 and 18 months in the soluble fraction from 5xFAD-Gal3 KO compare to the WT. The insoluble fraction showed a significant reduction in the Aβ40 and Aβ42 levels at 6 months in the 5xFAD-Gal3 KO mice compared to WT. A reduction of Amyloid Precursor Protein (APP) and APP cleavage products were found at 6 and 18 months, in 5xFAD-Gal3 KO mice. Immunohistochemistry confirmed a reduction in amyloid plaque deposition in the CA1 region of hippocampus in the 5xFAD-Gal3 KO at 6 months. Finally, in human AD brain sections, we were able to confirm the presence of Gal3 positive cells in association with senile plaques, corroborating our animal data.
Conclusions: We demonstrated that Gal3 is related with disease progression in the 5xFAD mouse model. A deficiency in Gal3 reduced the pro-inflammatory response, the amyloid plaque burden, the APP processing, in addition to altering the amyloid-beta CSF levels in this AD mouse model. Notably, Gal3 levels were up-regulated in correlation with the progression of the disease, and only microglial cells close to amyloid plaques showed Gal3 immunoreactivity. In summary, our findings suggest that Gal3 is a key molecule in AD pathogenesis and could be a potential therapeutic target in AD.

Disclosures: A. Boza-Serrano: None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.05/BB15

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NARSAD Young Investigator Grant (Schizophrenia)

Title: Mechanistic and structural studies of the double-hit maternal immune activation model: How microglia and neurons are involved in the progression of schizophrenia

Authors: *C. HUI, J. SAVAGE, M.-E. TREMBLAY;
Univ. Laval, Canada, QC, Canada

Abstract: Schizophrenia is a mental disorder affecting 0.4% of the population, with disease onset occurring in late adolescence or early adulthood. Patients have difficulty with working memory, attention, executive functions, and social interactions. There is emerging evidence from human studies that inflammation experienced prenatally is linked with increased risk for the development of neuropsychiatric disorders including schizophrenia. Moreover, changes of brain volume and increased inflammatory status are observed in schizophrenia patients. Polyinosinic:polycytidylic acid (Poly I:C), a toll-like receptor 3 agonist, is commonly used to mimic viral maternal immune activation in pregnant mice as a preclinical model of schizophrenia. Our recent work revealed that mice receiving a double-hit of Poly I:C, once prenatally at embryonic day (E)9.5 or E12.5 and once at postnatal day (P)30, show several behavioral deficits, including increased repetitive marble burying activity, enhanced anxiety in elevated plus maze, and reduced social interaction in the 3-chamber social novelty test.
To explain how the double-immune challenge leads to behavioral deficits, frontal cortex, hippocampus and cerebellum were harvested from animals 24-48 hours after the behavioral tests. Inflammatory status and expression of schizophrenia-related genes/proteins were investigated by qRT-PCR and western blot. Microglial and neuronal morphologies, cellular damage, microglial remodeling of neuronal circuits, and other modes of microglia-synapse interactions were assessed by confocal and electron microscopy. To determine the physiological activities of microglia in the brain, two-photon imaging was performed in live heterozygous CX3CR1-GFP mice after double Poly I:C challenge. By matching these biochemical and physiological results with previous behavioral findings, we hope to demonstrate how microglial-neuronal interactions are critically involved in the progression of schizophrenia and suggest novel therapeutic avenues for prevention and treatment.

Disclosures: **C. Hui:** None. **J. Savage:** None. **M. Tremblay:** None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

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Program#/Poster#: 516.06/BB16

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CDMRP Grant W81XWH-09-2-0098

Intramural Funds from Centers for Disease Control

Title: Corticosterone primes the inflammatory response to Gulf War Illness associated agent exposures in the brain but not the periphery

Authors: *A. R. LOCKER[1], K. A. KELLY[1], L. T. MICHALOVICZ[1], J. A. VRANA[1], Z. M. BARNES[2], M. FLETCHER[3], D. B. MILLER[1], J. P. O'CALLAGHAN[1];
[1]Mol. Neurotoxicology, CDC/NIOSH, Morgantown, WV; [2]Dept. of Med., Univ. of Miami, Miami, FL; [3]Inst. for Neuro Immune Med., Nova Southeastern Univ., Fort Lauderdale, FL

Abstract: Following the 1991 Persian Gulf War, 250,000 soldiers returned with symptoms of Gulf War Illness (GWI), a complex disorder with characteristics similar to chronic, persistent sickness behavior - a condition associated with neuroinflammation. Troops were exposed to a variety of conditions in theater, including physiological stressors (e.g., high temperatures, exercise, physical threat) and organophosphate (OP) acetylcholinesterase inhibitors (AChEI) in the form of insecticides (e.g. chlorpyrifos) or the nerve agent, sarin. Previously, we have shown that exposure to the AChEIs, chlorpyrifos-oxon (CPO) and diisopropyl fluorophosphate (DFP, a sarin surrogate), produces neuroinflammation, an effect that is augmented by pretreatment with the stress hormone, corticosterone (CORT) - a stress mimic. Here, we investigated the peripheral effects of exposure to CORT and DFP to determine if systemic inflammatory effects would align with the previously observed neuroinflammatory effects. We assessed peripheral inflammation by measuring cytokine levels in the liver and serum. We also investigated whether AChE activity predicted inflammation in the brain or periphery. Adult male C57BL/6J mice were exposed to CORT (either 200mg/L or 400mg/L) in their drinking water, followed by exposure to a single i.p. dose of DFP (4.0mg/kg). Unlike our previous results in the brain, exposure to DFP alone or with CORT did not result in the increased expression of inflammatory markers in the serum or liver. Interestingly, brain and blood AChE inhibition was not predictive of the neuroinflammation seen in mice exposed to CORT and DFP. In fact, CORT modestly ameliorated the AChE inhibiting effects of DFP, suggesting that neuroinflammation is not the result of AChE inhibition. This paradigm models veteran exposure in theater, but mice exposed to this initial paradigm followed by multiple waves of CORT and an inflammatory challenge with lipopolysaccharide (LPS - a bacterial mimic) more closely models the circumstances surrounding GWI. Exposure to this paradigm revealed that inflammation was increased in both

brain and blood. Unlike the brain, there was no difference in the levels of blood cytokines between mice exposed to CORT or CORT and DFP prior to the LPS challenge. Our results suggest that the persistent sickness behavior symptoms of GWI are a result of neuroinflammation, not peripheral inflammation, induced by a key combination of exposures (e.g., CORT and DFP). Moreover, the identification of blood markers for GWI may not be indicative of the neuropathobiology of the illness. Future treatments of GWI should focus on ameliorating neuroinflammation, a major contributor to GWI.

Disclosures: **A.R. Locker:** None. **K.A. Kelly:** None. **L.T. Michalovicz:** None. **J.A. Vrana:** None. **Z.M. Barnes:** None. **M. Fletcher:** None. **D.B. Miller:** None. **J.P. O'Callaghan:** None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

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Program#/Poster#: 516.07/BB17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Project PTDC/NEU-OSD/0312/2012

Pest-C/SAU/UI3282/2013-2014

Pest CNC.IBILI FCT-UID/NEU/04539/2013

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Title: Central effects of chronic methylphenidate treatment in control and hyperactive rats: neuroinflammation and blood-brain barrier

Authors: *V. COELHO-SANTOS[1,2,3], F. L. CARDOSO[1,2,3], R. A. LEITÃO[1,2,3], C. FONTES-RIBEIRO[1,2,3], A. P. SILVA[1,2,3];
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Abstract: Methylphenidate (MPH) is the most commonly prescribed drug for the treatment of attention deficit hyperactivity disorder (ADHD). Despite the widespread use of this psychostimulant, the cellular impact of its use is still largely unknown. Thus, the aim of the present study was to clarify the effect of MPH on cortical immune surveillance and blood-brain

barrier (BBB) properties in both physiological and ADHD conditions. For that, we used a rat model of ADHD, the Spontaneously Hypertensive (SHR) rats, as well as Wistar Kyoto (WKY) as inbred comparator strain. Also, to mimic a clinical dosing regimen in ADHD, rats were administered for Monday to Friday with vehicle or MPH (1.5 or 5 mg/kg/day, per os) during 4 weeks (P28-P55).
We concluded that 1.5 mg/kg/day of MPH downregulated both glial fibrillary acidic protein (GFAP; astrocytic marker) and ionized calcium-binding adapter molecule 1 (IBA1; microglia marker) in hyperactive rats. On the contrary, in WKY rats, both MPH doses increased GFAP levels, led to perivascular astrogliosis, and microgliosis. Neuroinflammatory events are known to perturb the BBB integrity, which is a multicellular vascular structure that separates the brain from the peripheral blood circulation maintaining an environment that allows neural cells to function properly. Thus, BBB dysfunction can lead to immune cell infiltration and brain injury. Herein, we observed that 5 mg/kg/day MPH increased BBB permeability in both WKY and SHR animals; however, more prominent in WKY rats. This effect can be explained by the downregulation of the tight junction protein, claudin-5, and disruption of the cerebrovascular basal lamina protein, collagen-IV. Noteworthy, WKY animals also showed an increase in the expression of caveolin-1, which is a structural protein required for caveolar transcytosis, and both vascular cell and intercellular adhesion molecules (VCAM-1 and ICAM-1), important proteins to promote adhesion and transmigration of peripheral cells into the brain. As a consequence of BBB alterations, CD169+macrophages were significantly identified in the brain parenchyma.
Overall, we demonstrated that chronic exposure to MPH under physiological conditions promotes neuroinflammatory events and brain vascular alterations. Interestingly, in the ADHD animal model, MPH at the lower dose had an anti-inflammatory. Our conclusions highlight the importance of an appropriate MPH dose regimen for ADHD, and the negative impact of MPH misuse.

Disclosures: **V. Coelho-Santos:** None. **F.L. Cardoso:** None. **R.A. Leitão:** None. **C. Fontes-Ribeiro:** None. **A.P. Silva:** None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.08/BB18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Recurrent coccidiodal meningitis treated with intrathecal administration of liposomal Amphotericin B

Authors: *B. FIANI, A. NGUYEN, D. NACIONALES, A. SODHI, G. FISCHBERG;
Desert Regional Med. Ctr. - ICON, Palm Springs, CA

Abstract: Background: Coccidiodal meningitis is a fungal infection that effects the meninges of the brain and is common to the southwestern United States and northwestern Mexico. Fluconazole is the accepted medical therapy, along with, amphotericin B.[1] Previous literature supports the use of intrathecal or IV liposomal amphotericin B in animal studies, but the importance of its usage in humans is evidenced by this case.[4] **Case Presentation:** A 44 year old Hispanic male presented with a past medical history significant for Coccidiodal meningitis in 10/2014 with recurrence. On physical exam, he was GCS 9 (M4 V1 E4), non-verbal, and did not cooperate with commands or physical exam, including testing Kernigs or Brudzinski sign. He symmetrically withdrew in all 4 extremities to painful stimuli. CT head showed diffuse ventriculomegaly. Enlargement of all ventricles. Hydrocephalus. No etiology identified. An EVD was placed for diagnostic and therapeutic purposes. CSF studies showed: 1,122 RBC, 186 WBC (21% neutrophils, 76% lymphocytes), 9.7 Coccidi IgG, 1:32 Coccidods Ab Qn, glucose 45 (serum glucose 99), protein 2,152. The patient was treated with intrathecal administration of liposomal amphotericin B thirteen times throughout the course of stay, 1.5 mg followed by 2 mL of sterile saline each time. Patient was also increased from fluconazole 400 mg to fluconazole 800 mg IV. Serial CSF studies were drawn before each administration of amphotericin B. Patient's labs and clinical picture improved from treatment. **Discussion:** The importance of this case comes from its applicability to show the effectiveness of intrathecal liposomal amphotericin B as a treatment method. CSF studies that were routinely collected trended in a promising direction toward improvement. In this patient, the use of intrathecal liposomal amphotericin B decreased CSF protein and CSF WBC including lymphocyte percentage. **Conclusion:** The above is a case of Coccidiodal meningitis in the setting of severe altered mental status and new-onset seizure. This patient shows the results of treatment with intrathecal administration of liposomal amphotericin B which supports prior animal studies and is breakthrough evidence in humans.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 NS085426

DoD/CDMRP W81XWH-14-1-0605

Craig H. Neilsen Foundation

Title: Pharamcologically inhibiting soluble tumor necrosis factor α signaling mitigates autonomic dysreflexia and improves cardiovascular function after a complete high thoracic spinal cord injury

Authors: *E. MIRONETS[1], S. HOU[1], J. R. BETHEA[2], P. OSEI-OWUSU[3], V. J. TOM[1]; [1]Neurosci., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Biol., [3]Pharmacol. and Physiol., Drexel Univ., Philadelphia, PA

Abstract: A consequence of severe, high-level spinal cord injury (SCI) is autonomic dysreflexia (AD), a life-threatening syndrome characterized by extreme, sudden bouts of hypertension triggered by sensory stimuli caudal to the injury. AD develops partly due to aberrant plasticity of sensory circuits caudal to the injury that lead to hyperactivation of sympathetic preganglionic neurons and hypertension. Since the pro-inflammatory, "master regulator" cytokine soluble tumor necrosis factor α (sTNFα) has been correlated with hyperexcitability, we hypothesize it plays a crucial role in this plasticity. We found that 4 weeks after a complete T3 transection (Tx) in adult rats, which virtually always results in AD, TNFα levels in lumbar cord are significantly higher than in naïve tissue, indicative of chronic inflammation in tissue caudal to a SCI. To test whether sTNFα is key for AD development, after T3 Tx, animals continuously received XPro1595, a biologic that inhibits sTNFα signaling, or saline intrathecally to spinal cord caudal to the SCI for 4 weeks. Notably, these rats had radiotelemeters implanted into the descending aorta at least 1 week prior to Tx to allow us to measure blood pressure (BP) and heart rate (HR) in the same rats at multiple time points. At 2, 3 and 4 weeks post-Tx, we assessed BP and HR before, during, and after colorectal distension (CRD) to trigger AD. Both groups of rats had similar basal BP. CRD induced sharp spikes in BP in saline rats that persisted long after CRD was alleviated. XPro1595 rats had significantly smaller and shorter hypertensive episodes during and after CRD. We also determined the number of spontaneous AD events that occurred over a 24-hour period at 2, 3, or 4 weeks. XPro1595 rats had significantly fewer spontaneously occuring events at all 3 testing points than saline animals. These data show that inhibiting sTNFα in caudal spinal cord dramatically diminished AD. This may be due to significantly less sprouting of $CGRP^+$ nociceptive afferents in dorsal horn and lamina X observed in the XPro1595 rats. To determine if inhibiting spinal sTNFα signaling improves peripheral vasculature function after SCI, we harvested mesenteric artery 4 weeks after Tx for ex vivo analysis. Vessels from saline-treated T3Tx rats had increased reactivity to vasopressors K^+ and phenylephrine compared to those from both naïve and XPro1595-treated T3Tx rats. Importantly, vessels from XPro1595 rats reacted similarly to naïve vessels. These data indicate that spinal sTNFα after SCI plays a critical role in plasticity leading to AD and altered peripheral vasculature, elucidating a potential target to diminish AD and improve cardiovascular health after SCI.

Disclosures: E. Mironets: None. **S. Hou:** None. **J.R. Bethea:** None. **P. Osei-owusu:** None. **V.J. Tom:** None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.10/CC2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: A National Science Foundation Graduate Research Fellowship

Title: Intrauterine inflammation leads to sex-dependent alterations in CNS inflammatory responses, and sex-independent changes in behavior

Authors: *R. A. MAKINSON[1], S. MCKEE[2], N. GRISSOM[1], B. GEORGE[2], M. MARINI[2], A. RAYASAM[3], Z. FABRY[3], T. REYES[1];
[1]Univ. of Cincinnati, Cincinnati, OH; [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Early life is a critical period in brain development, and adverse events during this time can have lasting effects on brain function and behavior. A common event that can disrupt normal brain development is exposure to inflammation *in utero*. In models using systemic maternal immune activation, a number of adverse effects on the brain and behavior have been documented. Chorioamnionitis, or the infection of the fetal membranes, occurs in approximately 10-15% of term births and is the focus of the current studies. Specifically, the goal is to investigate the extent to which intrauterine inflammation adversely affects the brain and behavior in male and female offspring. To model IUI, we utilize an injection of lipopolysaccharide (LPS), a bacterial mimic that induces an innate inflammatory response, directly into the uterus at embryonic day 15 in mice. We show that IUI significantly alters a number of parameters associated with proper brain development, behavior and immune activation. Specifically, IUI exposure decreased myelin basic protein (MBP) mRNA and protein, as assessed by immunohistochemistry and qPCR. Sex differences in assessed end points were also found. Specifically, the central nervous system's immune response to an acute LPS injection in adult males was potentiated, an effect absent in the female offspring. Males exposed to IUI exhibited a greater increase in interleukin-1beta, tumor necrosis factor alpha, C-X-C motif ligand 10 and suppressor of cytokine signaling 3. IUI also significantly increased the total number of microglia in both males and females. Lastly, current studies are underway to test whether IUI alters cognitive performance in the 5 choice serial reaction time task, which assesses executive function (e.g., attention and impulse control). These results indicate that IUI negatively impacts neurological development in a sex-dependent matter, and these effects persist into adulthood.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.11/CC3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Dynamic PET imaging with arterial input function of lipoposysaccharide induced neuroinflammation in rat

Authors: *J. RYTKÖNEN[1], P. POUTIAINEN[2,3], L. TOLPPANEN[1], A. NURMI[1], T. HUHTALA[1];
[1]Charles River Discovery, Kuopio, Finland; [2]Univ. of Eastern Finland, Kuopio, Finland; [3]Kuopio Univ. Hosp., Kuopio, Finland

Abstract: Neuroinflammation is associated with neurodegenerative diseases, including multiple sclerosis (MS), Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), and traumatic brain injury (TBI). Activation of the mitochondrial translocator protein (TSPO) in neuronal tissue is linked with neuroinflammation, thus TSPO ligands can be applied to image the progression of neuroniflammation *in vivo* using PET imaging. ^{18}F-FEPPA is second-generation TSPO ligand with improved BBB penetration, selectivity and suitable metabolic profile, with hydrophilic metabolites, which are less likely to penetrate the blood brain barrier. ^{18}F-FEPPA has been used in clinical PET imaging of neuroinflammation e.g. in PD, AD and schizophrenia. Dynamic PET imaging of neuroinflammation in rodent models is challenging due to several factors. TSPO upregulation is uniform in most many neurodegenerative diseases with neuroinflammation. This limits the use of reference tissue models, as no valid reference tissue is available. Traditional techniques for blood sampling to generate full arterial input function (AIF) are not feasible due to limited blood volume of rodents. Significant blood loss may also bias tracer pharmacokinetics. Furthermore, multiple blood sampling can be conducted only as a terminal procedure preventing longitudinal studies within individual. With arteriovenous shunt and coincidence counter blood input function can be obtained during dynamic PET scan without blood loss. Correction for plasma activity and parent fraction can be achieved from minimal blood volume samples collected from the shunt during the scan. This nonsurgical novel approach requires only minimal blood sampling allowing longitudinal PET studies with AIF in rats. Lipopolysaccharide (LPS) is a known potent immunostimulant, which can be used to induce acute local neuroinflammation after intracranial stereotactic infusion. In this study dynamic PET imaging of ^{18}F-FEPPA was performed after unilateral infusion of LPS to rat striatum. Dynamic 90 min PET scan was performed and blood activity was measured simultaneously from the shunt using coincidence counter. After imaging brains were collected for additional autoradiography and histological analysis. As a summary, applying this novel methodology to generate AIF

simultaneously with pre-clinical PET imaging provides more translational and reliable approach to monitor progression of inflammation applicable for several neurodegenerative rodent models.

Disclosures: **J. Rytkönen:** None. **P. Poutiainen:** None. **L. Tolppanen:** None. **A. Nurmi:** None. **T. Huhtala:** None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

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Program#/Poster#: 516.12/CC4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: MH64570

Title: Intercellular adhesion molecule-5 (ICAM-5) facilitates a unique and dynamic relationship between CD4+ t cells and hippocampal neurons during HIV-associated neurocognitive disorder (HAND)

Authors: ***A. J. TRZECIAK**[1], J. HAMMOND[2], S.-M. LU[2], H. GELBARD[3];
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Abstract: Neuroinflammation is often an unwanted side effect of many inflammatory disorders and peripheral infections. In the case of HIV-1 infection, circulating T cells are known to infiltrate the brain and cause neuronal damage and gliosis. In an effort to more closely examine some of these interactions, an *in vitro* rodent hippocampal model of HIV-associated neurocognitive disorder (HAND) was created using a well-known inflammatory mediator of HIV-1 infection known as platelet-activating factor (PAF), in addition to CD4+ T cells isolated from rodent splenocytes with a cocktail of anti-CD3ε, anti-CD28, rIL-2, then supplemented with additional IL-2 and co-cultured with DIV18 neurons. We specifically focused on the expression of a homing molecule in telencephalic neurons called intercellular adhesion molecule-5 (ICAM-5) and its unique ability to both recruit and suppress T cell function via interactions with its cognate receptor, leukocyte function antigen type 1 (LFA-1). We used the carbamyl congener of PAF (cPAF) because it is resistant to degradation by tissue acetylhydrolases. We found that over a 2log difference in cPAF concentration (100nM-10μM), there was no effect on neuronal viability, but subsequent co-culture with CD4+ T cells in increasing numbers demonstrated an inverse relationship between the transmembrane and soluble forms of ICAM-5. These findings indicate that neurons express intracellular ICAM-5 during PAF-induced toxicity, but switch to a secreted form of the molecule when CD4+ T cells are added at higher ratios, suggesting that

soluble ICAM-5 may be an "SOS" signal from neurons during HIV-1 infection of the CNS with increased CD4+ T cell trafficking into the CNS. The translational relevance of these findings is underscored by previous data from our lab that demonstrate a striking increase of soluble ICAM-5 seropositivity in serum from patients with HIV-1 infection, suggesting potential alterations in blood-brain barrier integrity and/or lymphocyte trafficking from the CNS to the periphery with bound soluble ICAM-5. We are extending these studies to assess whether there are additional effects of this type of signaling on adaptive immune cell populations including Tregs. Support: MH64570

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

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Program#/Poster#: 516.13/CC5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: This study was supported by the Drs. Chantal and Peritz Scheinberg Research Fund.

Title: Inflammasome activation exhibits sex difference and estrogen receptor beta agonist treatment reduces its activation and protects the brain from ischemic damage

Authors: *N. D'ADESKY[1], M. SCHATZ[2], M. A. PEREZ-PINZON[2], H. BRAMLETT[2], J. DE RIVERO VACCARI[2], A. P. RAVAL[2];
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Abstract: Cerebral ischemia is known to activate the innate immune response, and the inflammasome is a key component of the innate immune response. The inflammasome is comprised of caspase-1, the adaptor protein apoptosis associated speck-like protein containing a CARD (ASC), and a pattern recognition receptor such as a NOD-like receptor. Prior literature suggested that the inflammasome proteins are regulated by sex steroids. In a published study, we reported that the silencing of estrogen receptor subtype beta (ER-β) attenuated 17β-estradiol mediated decreases in caspase-1, ASC and interleukin-1β (IL-1β). Based on this information, the goal of the current study was to investigate whether inflammasome proteins alter with age and/or sex in the hippocampus and if these differences could contribute to increases in damage due to cerebral ischemia. We hypothesize that inflammasome activation is significantly higher in the hippocampus of reproductively senescent (RS) females as compared to their young counterparts and age-matched males. We also hypothesize that periodic pretreatment of ER-β agonist will reduce both inflammasome activation and cerebral ischemic damage in RS female rats. We

tested our hypothesis by investigating inflammasome protein levels in the hippocampus of young (6-7 month old) and reproductively senescent (11-13 month old) female Sprague-Dawley rats and age matched males. To test the efficacy of ER-β agonist on reducing inflammasome proteins and ischemic brain damage, ER-β agonist (1 mg/kg; every 48 h for 21 days) or vehicle (DMSO) treated RS females were sacrificed for collection of brain tissue or exposed to global cerebral ischemia. Results of western blot analysis demonstrated a significant increase in the inflammasome proteins caspase-1 ($p < 0.05$) and ASC ($p < 0.01$) in the hippocampus of RS females as compared to RS matched male rats. In the RS female periodic ER-β agonist pretreated group, we observed a significant decrease of the inflammasome proteins caspase-1 ($p<0.05$), ASC ($p<0.05$) and IL-1β ($p<0.05$) as compared to vehicle treated RS females. Furthermore, histological analysis of the hippocampus 15 days after global cerebral ischemia demonstrated a significantly higher number of live neurons ($p< 0.05$) in the ER-β agonist group as compared to vehicle treated RS female rats. Our findings suggest the role of sex hormones in the regulation of the inflammasomes in the hippocampus and that activation of ER-β could be useful in the prevention of post-ischemic inflammasome activation and reduction in ischemic brain damage.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

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Program#/Poster#: 516.14/CC6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Nuclear imaging of neuroinflammation in rodent models of neurodegenerative diseases

Authors: T. HUHTALA[1], J. RYTKÖNEN[1], P. POUTIAINEN[2,3], L. TOLPPANEN[1], A.-M. ZAINANA[1], T. PARKKARI[1], O. KONTKANEN[1], P. J. SWEENEY[1], *A. J. NURMI[1]; [1]Charles River Discovery, Kuopio, Finland; [2]Univ. of Eastern Finland, Kuopio, Finland; [3]Kuopio Univ. Hosp., Kuopio, Finland

Abstract: Neuroinflammation is associated with progression of various neurodegenerative diseases. Activation of the mitochondrial translocator protein (TSPO) is linked to neuroinflammation and TSPO ligands can be used for *in vivo* PET or SPECT imaging. ^{18}F-FEPPA is a second-generation TSPO ligand with improved penetration, selectivity and suitable metabolic profile. For *in vivo* SPECT/CT imaging of neuroinflammation, ^{123}I-CLINDE has been used as a TSPO radioligand. In the current studies, we utilized these ligands to assess the extent of neuroinflammation after lipopolysaccharide (LPS) infusion, following induction of multiple

sclerosis (MS) and neuropathic pain. LPS, a known potent immunostimulant, was used to induce acute local neuroinflammation. Dynamic PET imaging of ^{18}F-FEPPA after a unilateral infusion of LPS into the rat striatum was performed and blood activity was measured simultaneously from the shunt using a coincidence counter to generate the arterial input function (AIF) for kinetic modeling. To model MS, C57Bl/6 mice were given cuprizone (0.3% w/w) in their diet for 6 weeks. After SPECT/CT (^{123}I-CLINDE) and PET/CT (FDG) imaging had been performed, brains were dissected for autoradiography analysis and densities of cannabinoid receptor 1 (CB1) in treated and control animals were compared. A significant increase in accumulation of ^{123}I-CLINDE ($P < 0.05$) was observed in all studied brain regions, and a significant decrease in brain metabolism was revealed in several brain regions of cuprizone-treated animals compared to parameters measured in control mice. In addition, CB1 receptor density in the globus pallidus was significantly lower in cuprizone-treated mice than in control animals. Neuritis model was used to study perineural inflammation in rats. Induction was done using modified Complete Freund's adjuvant in Oxygel band wrapped around sciatic nerve. Animals exhibited neuropathic pain as well as five-fold accumulation of FDG on day 7, and 1.6-fold accumulation of CLINDE on day 4 post-induction was observed in the operated left hind leg. Persistent increase in metabolic activity was seen on day 7 and 21 post induction using FDG-PET imaging. As a summary, pre-clinical nuclear imaging provides more translational approach to monitor progression of inflammation and is applicable in several rodent models with neuroinflammation. In these studies, SPECT/CT imaging with ^{123}I-CLINDE was shown to effectively detect neuroinflammation. In addition, metabolic alterations associated with neuroinflammation were also quantified by using FDG/PET. Results highlight multiple options to study neuroinflammation in animal models.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS070261

Barrow Neurological Foundation

Title: Ketogenic diet-mediated K_{ATP} channels activation ameliorates neuroinflammation by intervening ketone body

Authors: H. OH[1], M. LEE[2], S.-C. MA[1], I.-H. CHO[1], *D. KIM[1];
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Abstract: Ketogenic diet (KD) is a proven therapy for patients with medically refractory epilepsy. There is also emerging data supporting its use beyond epilepsy in various neurological disorders (NDs). The metabolic shift towards fatty acid oxidation following the KD administration results in the eminent production of ketone bodies [KBs; β - hydroxybutyrate (BHB) and acetoacetate (ACA)] as a hallmark of metabolic change. While there is growing evidence suggesting that both the KD and KBs appear sufficient as disease-modifying actions in diverse NDs, the underlying mechanisms of this beneficial effect have yet to be elucidated. Our recent observation has provided that KBs negate neuroinflammation in glial cells and its effect may be relevant in control of ATP-sensitive potassium (K_{ATP}) channels (*SFN 2015, #121.14).* In the present study, we explored the functional relevance of K_{ATP} channels on the anti-inflammatory effect of either the KD or KBs under glial activation induced by lipopolysaccharide (LPS; an endotoxin) or rotenone (a mitochondrial respiratory complex 1 inhibitor) using immunohistochemistry, western blotting, mRNA expression, and CRISP shRNA. C57BL/6J mice were fed either the KD or a standard diet (SD) from postnatal 6 wks until 8 wks, and then LPS (3 mg/kg, intraperitoneally) was administrated to evoke glial cells activation. The KD significantly attenuated upregulation of tumor necrosis factor-α, interleukin-1 β (IL-1β) and IL-6 mRNA in hippocampi at 4 h after LPS injection. Morphological changes of microglia (Iba1 protein) and astrocyte (GFAP) by LPS were normalized with the KD. Hippocampal lysates collected from the KD-fed mice exhibited an increase in the inwardly rectifying K^+ channel subunit Kir6.2, a critical component of K_{ATP} channels, as compared to SD-fed mice. Under either LPS - or rotenone - induced glial cells activation, BHB had a strong reduction in the release of pro-inflammatory mediators and its action was linearly correlated with the downregulation of LPS-mediated M1 expression for pro-inflammatory cytokines or upregulation of IL4-induced M2 expression for anti-inflammatory cytokines. As expected, Kir6.2 immunoreactivity was significantly enhanced in both microglia and astrocyte exposed to BHB. The anti-inflammatory effects were counteracted by pharmacological blockers or shRNA of K_{ATP} channels. Collectively, our data indicate that both the KD and BHB protect against neuroinflammation. Furthermore, the underlying protective activity of both the KD and KBs influences either by enhancing anti-inflammatory properties and/or increasing K_{ATP} channels activation.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

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Title: MiRNA-22 regulates chronic inflammatory pain by positively targeting Mtf1

Authors: *Z. PAN[1], L.-Y. HAO[2], G.-F. LI[2], M.-L. SUN[2], L.-J. ZHU[2], Y.-D. LI[2], H.-L. DING[2], J.-L. CAO[2];
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Abstract: Increasing evidence has shown that miRNA negatively regulating expression of pain-related gene contributes to chronic pain, however, it remains unknown whether miRNA could positively gene expression in the modulation of chronic pain. Here, we found that complete Freund's adjuvant (CFA)-induced chronic inflammation pain significantly induced the increase of miRNA-22 expression in mice spinal neurons. Furthermore, Mtf1, a possible positive target of miRNA-22, was increased in the spinal cord of CFA mice. Knockdown of spinal miRNA-22 markedly attenuated pain behavior induced by CFA. Concurrently, the increased expression of spinal Mtf1 was reversed by miRNA-22 knockdown. In contrast, overexpression of spinal miRNA-22 in naive mice increased the expression of spinal Mtf1, and induced the production of pain-like behaviors, which could be inhibited by knockdown of spinal Mtf1 with siRNA. Collectively, we conclude that miRNA-22 regulates chronic inflammatory pain by positively targeting Mtf1.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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NIA

Title: VCAM1 is a mediator of age-related brain inflammation and decreased neurogenesis caused by an aged systemic milieu

Authors: *H. YOUSEF[1], C. CZUPALLA[2], J. ZANDSTRA[1], A. BURKE[1], H. HADEIBA[3], E. BUTCHER[2,4], T. WYSS-CORAY[1,4];
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Abstract: Studies from our lab and others have recently shown that brain function - specifically neurogenesis, synaptic plasticity and cognitive function in the hippocampus, a key center for learning and memory- is inhibited in young mice connected to aged mice through heterochronic parabiosis or aged plasma intravenous injections. While the identity of systemic inhibitory factors from aged plasma are beginning to be elucidated, the question of how and if these factors cross the blood brain barrier (BBB) and act directly on brain tissue to inhibit neurogenesis, or whether they act through their direct contact with brain endothelial cells (BECs) of the vasculature remain unanswered. BECs upregulate expression of vascular adhesion molecules as a result of increased systemic inflammatory signaling resulting from multiple diseases that afflict the CNS. We discovered that BEC-specific VCAM1 increases in the hippocampus during normal aging. Exposure of young BECs to an aged systemic environment induces BEC activation and upregulation of VCAM1 both in vitro and in vivo. Specifically, systemic injections of aged human blood into young immunodeficient (NSG) mice- acutely over 4 days or spread over 3 weeks- increased BEC-specific VCAM1 expression, increased brain inflammation as assessed by microglial activation, and inhibited hippocampal neurogenesis. Blocking VCAM1 signaling systemically with a neutralizing monoclonal antibody rescued neurogenesis and prevented aged plasma induced microglial activation. This study suggests that preventing BEC-immune cell crosstalk through VCAM1 may be a therapeutic target for ameliorating aged blood induced decline in brain function.
Funding: NIH and NIA
SA Villeda *et al*. *Nature* **477**, 90-94 (2011) doi:10.1038/nature10357
LK Smith *et al. Nature Medicine* **21**, 932-937 (2015) doi:10.1038/nm.3898

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

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Korean Ministry of Health & Welfare (Grant No. HI14C3331)

Title: A novel small-molecule agonist of PPAR-γ potentiates an anti-inflammatory phenotype in glia and endothelial cells

Authors: ***G. SONG**[1], Y. NAM[1], M. JO[1], J. KOO[2], S. PARK[2], K. SUK[1];
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Abstract: Neuroinflammation is a key process for many neurodegenerative diseases. Activated microglia and astrocytes play an essential role in neuroinflammation by producing nitric oxide (NO), inflammatory cytokines, chemokines, and neurotoxins. Therefore, targeting glia-mediated neuroinflammation using small-molecules is a potential therapeutic strategy. In this study, we performed a phenotypic screen using microglia cell-based assay to identify a hit compound (SNU-BP), which inhibits lipopolysaccharide (LPS)-induced NO production in microglia. SNU-BP inhibited pro-inflammatory cytokines and inducible nitric oxide synthase in LPS-stimulated microglia and astrocytes, and potentiated interleukin-4-induced arginase-1 expression. PPAR-γ was identified as a molecular target of SNU-BP. The PPAR response element reporter assay revealed that SNU-BP specifically activated PPAR-γ, but not PPAR-δ or -α, confirming that PPAR-γ is the target protein of SNU-BP. The anti-inflammatory effect of SNU-BP was attenuated by genetic and pharmacological inhibition of PPAR-γ. In addition, SNU-BP has anti-inflammatory effects in endothelial cells as well, by inhibiting LPS-induced MCP-1 and VCAM

expression. Finally, SNU-BP exhibited an anti-inflammatory effect in the LPS-injected mouse brain, demonstrating a therapeutic potential for neuroinflammatory diseases.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH T32 GM075770

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Title: Skin overexpression of neurturin increases antigen presenting cells recruitment to the skin and dorsal root ganglia and provides resistance to C. albicans infection

Authors: ***M. E. RITTER JONES**[1], C.-Y. LUI[2], C. YAO[5,6], B. M. DAVIS[2], D. H. KAPLAN[3,4], K. M. ALBERS[2];
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Abstract: Tissue inflammation and nerve injury can lead to chronic neurogenic inflammation and pain. Neurogenic inflammation involves cytokine release from nerve endings, both locally (at site of injury) and centrally, which induces vasodilation and further activation of the immune system. Changes in neurotrophic growth factors, which are known to increase sensory neuron activity, also occur in response to injury and inflammatory challenge. However, the exact role of these factors in modulating the inflammatory milieu at the site of injury remains to be elucidated. In recent studies, we determined that the growth factor neurturin (Nrtn) may regulate immune cell infiltration. In mice that overexpress Nrtn (Nrtn-OE) in the skin, an increased density of major histocompatibility complex II positive (MHC II+) antigen presenting cells (APCs) was observed in both the dorsal root ganglia (DRG) and skin when compared to wild type (WT) mice. We then tested if this Nrtn-induced increase in APCs altered the response to an inflammatory challenge elicited by a *Candida albicans* (CA) cutaneous infection. At 3 days post infection, the infected skin and DRG were isolated and analyzed by immunohistochemistry to detect MHC II+ cells. Colony forming assays (CFUs) were also done to assess the clearance of CA from infected skin. CFU assays showed significantly fewer colonies in Nrtn-OE skin

indicating enhanced CA clearance. Immunohistochemistry demonstrated that, in both the skin and DRG, there were a greater number of MHC II+ cells in NrtnOE mice. In the DRG, MHC II+ labeled cells appeared preferentially adjacent to neuronal cell bodies. The increase in Nrtn and APCs was associated with faster clearance of *C. albicans* and suggests a new role for Nrtn as a modulator of inflammatory cell infiltration and the innate immune system response.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Unfolded protein response and iron metabolism interplay in the CNS

Authors: *S. HEALY, J. MCMAHON, U. FITZGERALD;
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Abstract: The unfolded protein response (UPR), a ‘check-and-balance’ program activated upon disturbed homeostasis in the endoplasmic reticulum (i.e. ER stress), has been linked with iron homeostasis. Given that aberrant iron metabolism and increased expression of markers of the UPR have been independently reported in multiple sclerosis, Alzheimer’s disease and Parkinson’s disease, we believe that the crosstalk between UPR signaling/iron homeostasis might participate in neurodegeneration and that clarifying this relationship in the CNS will provide further clues into disease pathogenesis.
For the first time, using our novel *ex vivo* slice brain slice culture model of iron mismanagement, we demonstrate that 5 μg/ml tunicamycin exposure, which activates the UPR, ameliorates the toxic 1.6-fold iron accumulation produced by one μM ferrocene exposure ($P < 0.05$) suggesting a protective mechanism. Given that their interplay is reciprocal, the effect of iron dyshomeostais and UPR activation on each other was investigated both independently and together in our slice culture system.
By activating the UPR with tunicamycin, we investigated whether the CNS activation

recapitulates the iron dyshomeostasis (i.e. effects on hepcidin, ferroportin and ferritin-H expression) reported in UPR-activated hepatocytes. Building on this preliminary profile, we have also assessed changes in the transcripts of 14 key iron homeostasis molecules in our UPR-activated brain slices and also a subset of these molecules at the cellular level using immunohistochemistry and confocal microscopy.
Using real-time PCR, we have also identified and comprehensively characterized the effects of iron on the transmembrane sensors, ATF6 and PERK, the resident UPR chaperones, BiP and calreticulin, and also the proteins, CHOP and XBP1. These findings were confirmed at a cellular level where activated nuclear ATF6 was observed in the cell nuclei in iron-loaded slice cultures. In summary, we have comprehensively characterised the iron-UPR interplay in the brain and believe this research provides groundwork for understanding the role this relationship plays in neurodegenerative disease.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

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Title: Using a novel hybrid enzyme to identify the mechanisms of paracrine and autocrine effects of the inducible COX-2 and mPGES-1-produced prostaglandin E_2 on mouse hippocampal neurons

Authors: *Q. LING, H. AKASAKA, E. MURDOCH, K.-H. RUAN;
Univ. of Houston, Houston, TX

Abstract: Nonsteroidal anti-inflammatory drugs (NSAIDs) are specific inhibitors of cyclooxygenase (COX)-1 and COX-2, which mediate metabolism of arachidonic acid (AA). Their effects in heart and vascular diseases have been well established, but how they regulate neuronal inflammation and neurodegeneration is still not clear. In order to address this question, we have investigated both paracrine and autocrine effects of the NSAIDs-targeted AA-metabolites, prostaglandin E_2 (PGE_2), prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) on

mouse hippocampal neurons. First, to study the paracrine effects, HT22 cells, the hippocampal neuronal cell line, were co-cultured with the HEK293 cells over expressing our created hybrid enzymes (COX-2-10aa-mPGES-1, COX-1-10aa-PGIS, and COX-1-TXAS) to specifically direct the AA metabolites to PGE_2, PGI_2 or TXA_2, respectively. The PGE_2 produced by COX-2-10aa-mPGES-1 and secreted from the HEK293 cell exerted cell damage on HT22 cells, compared to that of the HEK293 cells secreted PGI_2 and TXA_2. These data demonstrated that PGE_2 mediated neuronal inflammation could be through paracrine mechanism. Next, to test the autocrine effect of the PGE_2, we transfected HT22 cells with the cDNA of the hybrid enzymes and redirected their cellular AA metabolites to the corresponding prostanoids. When challenged by amyloid beta peptide (residues 25-35), the PGE_2 produced by COX-2-10aa-mPGES-1 and released by the neuron cells caused self-damages and death, similar to that of the paracrine effects. These data indicated that autocrine effect of the inflammatory PGE_2 on the neuronal cells. The study has provided novel evidences that neuronal inflammation and degeneration could be exerted by the inflammatory PGE_2 produced by the neuron cell self and the surrounding microglia cells through both autocrine and paracrine effects. Additionally, we incubated HT22 cells with analogues of PGE_2, which also induced cellular damages, leading to cell death. These effects were blocked by specific EP1 antagonist SC-19220. The identified EP1 involved in mediating the PGE_2 singling on the neuronal inflammation could be a potential target for the anti-neuronal inflammation and degeneration. As a support, we are going to investigate the genes that are involved in the EP1-mediated neuronal inflammation and degeneration within the neuron cells.

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Poster

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIAAA

Title: Hmgb1 and il-1β heterocomplexes in brain increase neuroimmune responses

Authors: *L. G. COLEMAN, JR[1], F. T. CREWS, 27599[2], J. ZOU[2];
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Abstract: Introduction: Innate immune responses involve release of classical innate immune molecules such as HMGB1 and IL-1β. The brain is rich in these molecules and neuroimmune

activation has been implicated in many disease states. Microglia are the primary mediator of neuroimmunity and release HMGB1 in response to ethanol. HMGB1 is also known to act as a chaperone for certain cytokines, enhancing their action at their receptors. **Methods**: *In Vivo:* Adult C57/BL6 mice were treated with ethanol (6g/kg, i.g.). Brain, plasma and lung were collected and analyzed at 6, 12, 18, 24, and 48 hours after ethanol. *In Vitro:* BV2 microglia were exposed to ethanol for 6-24 hours. Lysates and media were analyzed for HMGB1 protein. Hippocampal-entorrhinal brain slice culture (HEC) was used to assess the immune actions of HMGB1/IL-1β heterocomplexes. Recombinant HMGB1 and recombinant IL-1β were at 4C for 24 hours to form HMGB1/IL-1β complexes for functional experiments. **Results**: *In Vivo:* We found that HMGB1 and IL-1β were stored in the brain and lung in heterodimeric complexes in addition to free forms. Acute high dose ethanol resulted in a transient increases in HMGB1 in the brain (95% increase at 1 h), plasma (119% increase at 12 h), and lung (171% at 6 hours). Western blot and co-immunoprecipitation revealed that HMGB1 and IL-1β were stored in the brain together as a heterocomplex. *In Vitro*: HMGB1/IL-1β heterocomplex were also found in BV2 microglia. However, they were not present in SH-SY5Y neurons. Acute ethanol (100mM for 24 hours) increased the amount of HMGB1/IL-1β heterocomplexes in BV2 microglia (4-fold). HMGB1/IL-1β heterocomplexes were hyper-inflammatory in HEC brain slice culture. HMGB1 (17mM) alone did not induce iNOS. IL-1β alone (600pM and 6nM) increased iNOS by 712% and 2981% respectively. HMGB1/IL-1 complexes increased iNOS expression by 1.7- 2-fold above IL-1β alone (1452% and 4941% at 600pM and 6nM). IL-1β induced its own gene induction to 2367% and 11,621% of control (600pM and 6nM respectively). HMGB1/IL-1β complexes increased IL-1β gene induction by 1.7 to 3.5 fold above IL-1β alone (8379% and 19,916% above control, 600pM and 6nM IL-1β respectively). IL-1 receptor antagonist reduced the HMGB1/ILβ induction of iNOS by 64% while returning TNFα and IL-1β to control levels.**Conclusions**: HMGB1 and IL-1β are stored together as a heterocomplex in the brain in addition to their free forms. HMGB1/IL-1β complexes are more potent than IL-1β alone and act via enhancement of IL-1β signaling. Ethanol increases HMGB1/IL-1β heterocomplexes in microglia. Induction of neuro-HMGB1/IL-1β heterocomplex formation may be involved in the neuroimmune activation associated with alcoholism.

Disclosures: L.G. Coleman: None. **F.T. Crews:** None. **J. Zou:** None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.23/DD4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: European Union's Seventh Framework Program FP7 under grant agreement 607962 (nEUROinflammation)

Title: Scm-like with four mbt domains 2 cluster microRNA decreases neuronal survival in oxygen-glucose deprivation and reperfusion model and influences microglial inflammatory response *In vitro*

Authors: *N. KOLOSOWSKA[1], V. KEKSA-GOLDSTEINE[1], L. BRUNELLO[1], S. LOPPI[1], N. BISTER[1], P. KORHONEN[1], I.-L. KOLARI[2], T. TURUNEN[2], M. TURUNEN[2], K. KANNINEN[1], J. KOISTINAHO[1], T. MALM[1];
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Abstract: Stroke is known to induce a proinflammatory response leading to increased cell death and exacerbated secondary injury in the brain. We have shown that interleukin-33 (IL-33) administration is protective against ischemic insult by decreasing neuroinflammation and by shifting the microglial polarization towards M2 phase in the brains of Balb/cOlaHsd mice subjected to permanent middle cerebral artery occlusion (pMCAo).
The role of small, non-coding RNAs in regulation of gene expression in stroke is still emerging. We found out, that IL-33 treatment diminishes stroke-induced increase in Scm-like with four mbt domains 2 (Sfmbt2) cluster miRNA (referred to as Sfmbt2 miRNA) levels in the ipsilateral hemisphere of the brain 3 days after pMCAo. We carried out this study to investigate the influence of this particular miRNA on neuronal survival and microglial functions. Sfmbt2 miRNA levels were increased in mouse primary cortical neurons after glutamate treatment and in Neuro-2a exposed to 2 h oxygen-glucose deprivation and 24 h reperfusion (OGD/R), while microglial BV2 cells exposed to lipopolysaccharide (LPS) exhibited decreased levels of this miRNA. To study the gain-of-function of Sfmbt2 miRNA on neuronal survival and microglial properties, we stably transduced N2a and BV2 cells with lentiviral vector and thus drove Sfmbt2 miRNA overexpression. N2a viability following OGD/R exposure was measured by MTT assay. BV2 cells were treated with LPS, after which cytokine levels in the conditioned media were measured by the cytometric bead array, phagocytic activity by phagocytosis assay, nitric oxide (NO) production by the Griess colorimetric assay and protein levels quantified by Western blot. Sfmbt2 miRNA and inflammation-related gene expression levels were measured by qRT-PCR. The results show that overexpression of Sfmbt2 miRNA reduces N2a viability after OGD/R as compared to the cells expressing the control vector. After LPS exposure, production of proinflammatory cytokines by BV2 cells stably transduced with lenti-Sfmbt2 miRNA was decreased, as well as expression levels of proinflammatory factors, such as matrix metalloproteinase 9 (MMP-9) and tumor necrosis factor alpha (TNFα). Phagocytic capacity of Sfmbt2 miRNA transduced BV2 cells was decreased and these cells exhibited increased NO release. Protein levels of triggering receptor expressed on myeloid cells 2 (TREM2) were decreased in Sfmbt2 miRNA overexpressing BV2 cells compared to cells expressing the control vector. Our results suggest that in ischemic stroke Sfmbt2 miRNA may exhibit cell-specific actions resulting in decreased neuronal survival and dampened microglial phagocytic activity.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.24/DD5

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Methamphetamine causes differential gene expression in the cynomolgus monkey hippocampus according to age

Authors: ***S. BANG**[1], M. CHO[2], S. KWAK[3], Y.-B. JIN[4], Y. LEE[5], K.-J. JEONG[5], K.-T. CHANG[6], Y. CHAI[7], S.-R. LEE[8], D.-J. KIM[9];
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Abstract: Recently, methamphetamine (MA) use in adolescent has increased persistently and their exposure to MA at earlier ages compared to adults can cause more severe damage in the brain. It is necessary to evaluate the cellular and molecular mechanisms associated with brain damage induced by MA depending on the age. The present study was aimed to investigate the differentially expressed genes (DEGs) and their functions in the hippocampus of cynomolgus macaques, *Macaca fascicularis*, according to age after the administration of MA. Cynomolgus monkeys were divided as follows: Control, 6-12 months (age1, A1), 3-4 (A2) and 7-9 (A3)

years, and more than 11 years (A4). After the administration of MA in cynomolgus monkeys on an age-dependent dose (2 mg/kg, intramuscular injection in A1, A2, A3 and A4), transcriptome profiling in the hippocampus was performed using RNA-seq technology. The functions and networks of analyzed DEGs were classified using GO analysis tool (DAVID) and IPA software. Some genes were validated with real-time RT-PCR (RT-qPCR). In MA-treated animals, the correlation of DEGs between M1 and M4 was higher than other groups, showing the possibility that biological mechanisms in the hippocampus of M1 and M4 are similarly affected by METH. Based on the GO analysis using the DEGs, transmission of nerve impulse (GO:0019226) and synaptic transmission (GO:0007268) were significantly ranked within top three of GO biological process (BP) terms in all age monkeys exposed to MA compared to the control. As a result of RT-qPCR, expression patterns of FEZF1, GPR1N1, DLX1 and KCNIP2 mRNAs associated with neuron differentiation were similar to the results from RNA-seq. Our results suggest not only molecular mechanisms related to the DEGs in the impaired hippocampus but also a clue for developing MA addiction marker according to ages.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.25/DD6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: University of Florida Dept of Neurosurgery

Title: Inflammasome expression in the central nervous system.

Authors: *S. ADAMCZAK[1], J. KRESAK[2], M. RAHMAN[3];
[1]Univ. of Florida, Dept of Neurosurg., Gainesville, FL; [2]Dept. of Pathology, [3]Dept. of Neurosurg., Univ. of Florida, Gainesville, FL

Abstract: The innate immune system is the body's first line of defense against danger, either from pathogens or damaged host tissue. The central nervous system (CNS), once believed to be a victim of chemical warfare between pathogens and professional immune cells, is now recognized as an active player in the innate immune defense against not only infection, but also injured tissue and malignancy. However, the role of the innate immune system, specifically the inflammasome, in primary brain tumors is unknown. Here, we used immunohistochemistry to measure the expression levels of inflammasome proteins in 25 human glioma samples (both high

and low-grade.) We found that inflammasome proteins ASC, caspase-1, Pannexin- 1, and NALP-1 are expressed in human gliomas. The expression pattern is highly variable, suggesting important molecular differences among both high and low-grade gliomas. This is the first characterization of inflammasome proteins in human glioma samples, and the high expression levels suggest an important role for the inflammasome in primary CNS tumor biology. Further elucidating the role of inflammasome proteins in tumorgenesis may help direct treatment.

Disclosures: **S. Adamczak:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **J. Kresak:** None. **M. Rahman:** None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

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Program#/Poster#: 516.26/DD7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRSA NIH NINDS 1F31NS092230-01

Title: Inflammation decreases AMPA-mediated calcium permeability in striatal D2 but not D1 spiny projection neurons through L-type voltage-gated calcium channels

Authors: *C. WINLAND[1], N. WELSH[1], S. VICINI[2], K. MAGUIRE-ZEISS[1];
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Abstract: Neuroinflammation coincides with the progression of striatal neurodegenerative diseases. It is well established that activated microglia release proinflammatory molecules. In addition to perpetuating the innate immune response, these proinflammatory cytokines can also regulate synaptic plasticity through alterations of AMPA receptors (AMPARs). AMPARs are glutamatergic ion channels that can be calcium permeable or impermeable. The preferential trafficking of calcium-permeable versus impermeable AMPARs alter $[Ca^{2+}]_i$ which can have numerous consequences. Furthermore, voltage-gated calcium channels can be activated by small depolarizations, as in the case of AMPAR activation, and are also affected in proinflammatory conditions. Therefore, the present study sought to determine if a proinflammatory environment could alter AMPA-mediated $[Ca^{2+}]_i$ in dopamine-2 and dopamine-1 expressing spiny projection neurons (D2 and D1 SPNs). Lipopolysaccharide (LPS) was used as a model neuroinflammation. Acute striatal slices were incubated with LPS or vehicle for 2 hours prior to biochemical or imaging experiments. Microglial activation was confirmed by morphofunctional analyses. AMPA-mediated $[Ca^{2+}]_i$ was elicited with an AMPA cocktail containing AMPA, CPP, and

cyclothiazide with or without TTX and voltage-gated calcium channel blockers. Calcium imaging was conducted in slices prepared from Adora2a-GCaMP6 or D1-GCaMP6 transgenic mice. The percent of responding cells in both Adora2a-GCaMP6 and D1-GCaMP6 slices did not differ between the vehicle and LPS condition suggesting that LPS treatment did not result in direct toxicity. We found that LPS treated slices from Adora2a-GCaMP6 mice resulted in an increase of $[Ca^{2+}]_i$ without voltage-gated calcium channel blockade. In contrast, there were no changes in AMPA mediated $[Ca^{2+}]_i$ due to LPS treatment in D1-GCaMP6 slices without voltage-gated calcium channel blockade. Interestingly, when a nonspecific (i.e., cadmium) calcium channel blocker was included in the AMPA cocktail, we observed a decrease of AMPA-mediated $[Ca^{2+}]_i$ in D2 SPNs but not in D1 SPNs following exposure to LPS. Similarly, when a specific L-type voltage-gated calcium channel blocker (i.e., Isradapine) was included in the AMPA cocktail, we observed a decrease of AMPA-mediated $[Ca^{2+}]_i$ in D2 but not in D1 SPNs in the LPS condition. The findings of this study suggest that D2 SPNs may be more susceptible to proinflammatory conditions when compared to D1 SPNs and that this susceptibility is mediated in part by L-type voltage-gated calcium channels.

Disclosures: **C. Winland:** None. **N. Welsh:** None. **S. Vicini:** None. **K. Maguire-Zeiss:** None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.27/DD8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Lebanese National Council for Scientific Research

Title: Intracerebroventricular (icv) injections of endotoxin (ET) reduces hippocampal neurogenesis

Authors: L. BITAR[1], F. CHAMAA[1], B. SAFIEH-GARABEDIAN[2], E. AL-CHAER[1], N. SAADE[1], *W. ABOU-KHEIR[1];
[1]American Univ. of Beirut, Beirut, Lebanon; [2]Qatar Univ., Doha, Qatar

Abstract: Background: Constant formation of functional neurons from neural stem and progenitor cells in postnatal stages has been observed in two main neurogenic brain regions; the subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ) in the lateral ventricles. Adult neurogenesis, however, is prone to alterations by different physiological, pathological and pharmacological stimuli. One flaunting stimulus is neural inflammation which has been implicated in neurodegenerative disorders. Subsequently,

attempts to reverse inflammation using anti-inflammatory agents may serve as a possible intervention. Our aim was to demonstrate that inflammation, induced by *icv* injection of Endotoxin (ET) altered the behavior as well as the neurogenic niche of adult rats by decreasing neurogenesis.
Methods: Adult Sprague-Dawley male rats (250-300g) received stereotaxic *icv* injections of ET (6 µg in 1.5 µl) or sterile saline as described previously (Safieh-Garabedian et al., Neuropharmacology, 2011,60:496-504). Rats then received 3 injections (66mg/Kg/injection; *ip*) of 5'-bromo-2'-deoxyuridine (BrdU) and were perfused at different time intervals (Days 1, 2, 3, 6, and 9). The non-steroidal anti-inflammatory drug piroxicam® was given as daily injections to rats perfused at day 3. Behavioral pain tests were performed and BrdU positive cells were counted in the DG of the hippocampus.
Results: ET injection resulted in a significant decrease ($p < 0.0002$) of adult neurogenesis in rats at day 2 (439.75±81.16) and day 3 (479.875±94.69) when compared to sham (966.16±49.60). This was followed by an overshoot at day 6 (1124.80±161.18) then recovered the basal levels at day 9 (997±87.23). These alterations were accompanied by thermal hyperalgesia that peaked at day 3 (500%). Daily treatment with Piroxicam® (12.5 mg/kg; ip) was able to alleviate the ET effects on neurogenesis and reduce hyperalgesia.
Conclusions: The current study sheds light on the negative impact, reflected on neurogenesis in the hippocampal formation, of discrete neuro-inflammation which is a hallmark of neurodegenerative disorders. Thus, understanding the adverse effects of neuro-inflammation on neurogenesis opens a new window for the treatment and management of neuro-inflammatory disorders.
Keywords: Neuro-inflammation, Neurogenesis, Dentate Gyrus, Hippocampus
This research was supported by grants from the Lebanese National Council for Scientific Research

Disclosures: L. Bitar: None. **F. Chamaa:** None. **B. Safieh-Garabedian:** None. **E. Al-Chaer:** None. **N. Saade:** None. **W. Abou-Kheir:** None.

Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.28/DD9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Pilot Grant, Craig H. Neilsen Foundation

Empire Clinical Research Investigator Program

Title: Transcriptional profiling of circulating leukocytes in persons with chronic spinal cord injury

Authors: P. HERMAN[1], A. STEIN[2], K. GIBBS[1], *O. BLOOM[1,2];
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Abstract: Introduction: It is increasingly clear that persons living with traumatic spinal cord injury (SCI) have altered immune system responses, which include hallmarks of inflammation, immunosuppression, and autoimmunity (Schwab et al 2014). It has been proposed that these altered immune system responses may deleteriously impact functional recovery and promote infections, accelerated atherogenesis and other medical complications of living with SCI (Schwab et al 2014). Here, we investigated the hypothesis that the transcriptional profile of circulating leukocytes isolated from persons with chronic SCI would be altered as compared to uninjured persons.
Methods: This prospective, IRB-approved, observational study of adults with chronic (≥1 year from initial injury) SCI was performed in an academic medical center. RNA was isolated from whole blood from a PAXgene tube, using standard methods and the manufacturer's protocol (Qiagen QIAcube, Venlo, The Netherlands). RNA was amplified using Illumina RNA Total Prep Amplification Kit (Life Technologies, Carlsbad, CA) and analyzed on the Illumina Human HT-12v4 chip. Data was quantile normalized using Illumina's Genome Studio software and then analyzed for differential gene expression using Partek Genomics Suite (St. Louis, MO).
Results: Uninjured (N=26) and SCI (N=31) participants included 7 and 6 females, respectively. Participants in both groups were of similar ages (mean±sem, range): (48.3±2.3, 23-66 and 55.0±2.8, 21-80 years, $P<0.06$). Participants with chronic SCI were injured for 15.7±2.3 years (mean±sem). The most common etiologies of SCI were Fall (32%), Sports (32%), MVA (23%), and Other (13%). Spinal cord injuries occurred mostly above the level of T5 (74%) and 52% were neurologically complete (AIS A). We determined that there were 1693 genes that were differentially expressed (DE) in persons with SCI, with a false discovery rate (FDR) equal to 0.05. Gene set enrichment analysis was performed using the Enrichr platform (Chen et al 2013). Within the Kyoto Encyclopedia of Genes and Genomes (KEGG), WikiPathways, and Panther databases, DE genes were enriched in categories and pathways related to immune system function and metabolic signaling. Ongoing and future analyses will examine correlations between DE genes with level and severity of injury, as well as gender.

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.29/DD10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA

Title: IL1 and TNF signaling pathway inhibition reduces morbidity, mortality, neurodegeneration and convulsion incidence in mice following exposure to soman

Authors: *E. A. JOHNSON, J. F. IRWIN, K. LAITIPAYA, J. K. CHANDLER, D. D. PALMER, T. M. FERRARA-BOWENS, M. WEGNER, C. L. HONNOLD;
Pharmacol., US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD

Abstract: Exposure to organophosphorus compounds such as soman (GD) can initiate status epilepticus (SE) that can lead to progressive brain damage and behavioral impairment. GD, a potent acetylcholinesterase inhibitor and chemical warfare nerve agent, causes prolonged SE activity and cell death in the hippocampus, thalamus and piriform cortex. Current treatments can ameliorate GD-induced SE, though treatment effectiveness diminishes rapidly following exposure. Therefore, neuroprotective strategies that can be used at later time points are needed to reduce neurodegeneration and improve cognitive outcomes. One strategy involves modulation of the neuroinflammatory response, a prominent feature in GD-induced brain injury. We have previously shown that loss of TNF and IL-1 signaling can confer moderate neuroprotection after SE, though the absence of both has a synergistic effect. This study focused on inhibition of IL-1 and TNF signaling as a viable neuroprotective and anti-convulsant strategy and to reduce morbidity and mortality after GD-induced SE. Using a IL-1 receptor 1 (IL-1R1)/TNF receptor 1A (TNFR1A) double knockout (KO) mouse and background strain mice treated with inhibitors for IL-1 and TNF signaling, changes in neuropathology, mortality, seizure onset and other relevant physiological responses were compared to untreated wild type, IL-1R1 KO and TNFR1A KO mice. These results suggest that inhibition of multiple pathways is superior to treating individual inflammatory pathways to improve acute outcomes following GD-induced SE. Further studies will investigate the chronic neuroprotective and behavioral outcomes of these treatments. The views expressed in this talk are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. These studies were funded by the Defense Threat Reduction Agency (DTRA). This research was supported in part by an

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Poster

516. Inflammatory Mediator Function in Models of Neurodegeneration

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 516.30/DD11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH MH087332

NIH MH104131

NIH MH105330

NIH DA026306

Title: Nmda receptor-gated and L-type Ca^{2+} channels critically contribute to cxcl12-induced neurotoxicity.

Authors: ***M. KAUL**[1,2], K. MEDDERS[1,3], P. SANCHEZ-PAVON[1], D. OJEDA-JUAREZ[1], R. MAUNG[1], A. B. SANCHEZ[1];
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Abstract: The chemokine receptor CXCR4 (CD184) and its natural ligand CXCL12 contribute to many physiological processes besides cell migration, including decisions about cell death and survival in the central nervous system. Previously, we observed that CXCL12 can cause toxicity in cerebrocortical neurons in mixed neuronal-glial cell cultures via a pathway that required CXCR4 and the stress-associated mitogen activated protein kinase p38 (p38 MAPK). In the present study we show that blockade of NMDA type glutamate receptor-gated ion channels and L-type Ca2+ channels both prevent CXCR4-mediated toxicity of CXCL12 in cerebrocortical neurons. We also found that in cerebrocortical cells active/phosphorylated p38 MAPK is

primarily located in neurons, and CXCL12 increased the level of active of p38 MAPK. While blocking L-type Ca2+ channels with Nimodipine kept the active kinase at baseline levels, phospho-p38 MAPK was strongly reduced by blocking NMDA receptors with MK-801. Altogether, our findings linked CXCL12 induced neuronal death to regulation of cellular Ca2+ flux which is not only controlled by NMDA receptors but also by L-type Ca2+ channels upstream of p38 MAPK activation.

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Poster

517. Ischemia: Molecular Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 517.01/DD12

Topic: C.07. Ischemia

Support: NIH grant MH087823

Title: Rapid F-actin reorganization occurs in neurons during cytotoxic edema both *In vitro* and *In vivo*

Authors: ***B. CALABRESE**[1,2], H. ZHANG[2], J. T. AUNG[2], A. Y. SHIH[3], S. HALPAIN[2]; [1]Div. Biol, Sec Neurobiol, [2]UC San Diego, La Jolla, CA; [3]Med. Univ. of South Carolina, Charleston, SC

Abstract: Acute brain injury during stroke disrupts neural circuits, resulting in impaired cognitive and motor functions. Such injuries induce cytotoxic edema (pathological cell swelling), which is the primary mode of acute neuronal cell death in early post-ischemic stages. Cytotoxic edema is triggered by hyperactivation of voltage-gated ion channels or glutamate receptors. The capacity of neurons to cope with cellular edema is critical for cell survival; however little is known about the subcellular and molecular changes regulating this process. We find that a substantial reorganization of filamentous actin (F-actin) occurs during cytotoxic edema, both *in vitro* and *in vivo*. This reorganization is visualized using either fluorescently labeled phalloidin to detect F-actin directly, or by immunostaining for the actin binding protein drebrin. Following a localized photothrombotic stroke in mouse cortex, F-actin is lost from dendritic spines of pyramidal neurons and accumulates aberrantly within the soma and proximal dendrites. A similar redistribution of F-actin occurs in cultured hippocampal neurons within 15-120 min following oxygen-glucose deprivation and is blocked by NMDA receptor antagonists. Incubation of cultured neurons with either NMDA or the sodium channel activator veratridine induces this

F-actin reorganization within 4-5 min in 40-60% of neurons, with longer NMDA exposures recruiting larger fractions of neurons up to >95%. However, if NMDA receptor hyperactivation is limited to 5-10 min, neurons spontaneously recover their normal F-actin distribution within 1-2 hours and do not undergo cell death, suggesting that these actin changes may be pro-survival. Significant cell swelling accompanies the accumulation of F-actin within the somatodendritic domain, and the two phenomena share a similar time course and pharmacological profile, including a dependence on specific chloride ion influx pathways. Other forms of cellular stress do not trigger the same neuronal F-actin changes. We therefore postulate that the "actinification" of the somatodendritic compartment is specifically connected to cytotoxic edema, helping to preserve the neuron's structural integrity until ionic and osmotic homeostasis can be restored, and thereby limiting necrotic cell death.

Disclosures: **B. Calabrese:** None. **H. Zhang:** None. **J.T. Aung:** None. **A.Y. Shih:** None. **S. Halpain:** None.

Poster

517. Ischemia: Molecular Mechanisms

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 517.02/DD13

Topic: C.07. Ischemia

Support: NIH Grant NS046742

Title: The role of miR-34 in ischemia-induced neuronal death

Authors: *J.-Y. HWANG, F. PONTARELLI, B. L. COURT VAZQUEZ, R. ZUKIN;
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Abstract: Transient global ischemia arising as a consequence of cardiac arrest in humans causes selective, delayed death of hippocampal CA1 pyramidal neurons and cognitive impairment. Effective treatments to ameliorate the neurodegeneration and cognitive dysfunction associated with global ischemia are an unmet need. Emerging evidence points to a widespread role for microRNAs (miRNAs) as key modulators of target gene expression in neurons. Accordingly, dysregulation of miRNAs are implicated in the pathophysiology of neurodegenerative disease and neurological disorders. Our findings, derived *via* miRNA-seq, indicate that a subset of microRNAs is altered in postischemic CA1 including miR-34b/c. Dysregulation of miR-34 has been implicated in pathophysiology of neurological disorder such as Parkinson's disease and epilepsy. However, a role for miR-34 in the pathogenesis of global ischemia is, as yet, unclear. Here we show ischemia induces p53-dependent activation of miR-34b/c and downregulation of

its target genes, which together promote neuronal death in selectively vulnerable hippocampal neurons. These findings have great potential for our understanding of how global ischemia induces neuronal death and identify a novel therapeutic target for amelioration of the neurodegeneration and cognitive deficits associated with ischemic stroke.

Disclosures: J. Hwang: None. **F. Pontarelli:** None. **B.L. Court Vazquez:** None. **R. Zukin:** None.

Poster

517. Ischemia: Molecular Mechanisms

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Program#/Poster#: 517.03/DD14

Topic: C.07. Ischemia

Support: U01EB017695

NIH R01MH086638

Title: Mosaic multiscale computer modeling of ischemic stroke

Authors: *A. SEIDENSTEIN[1], W. W. LYTTON[2], R. A. MCDOUGAL[3], M. L. HINES[3]; [1]CBE, New York University- Tandon Sch. of Engin., Brooklyn, NY; [2]Physiol. and Pharmacol., SUNY-Downstate Med. Ctr., Brooklyn, NY; [3]Neurosci., Yale Univ., New Haven, CT

Abstract: Ischemic stroke produces oxygen and glucose deprivation in brain cells, leading to cell dysfunction and death. We employ computer modeling to provide a platform to stimulate cell death, ion dysregulation, and edema due to ischemia. In doing so, we create tools to understand damage across temporal and spatial scales and to suggest where there is potential for neuroprotection.

On a cellular level, molecular cascades produce damage that can lead to apoptosis, necrosis or necroptosis. Damage spreads due to a combination of bulk flow of glutamate, reactive oxygen species, and other agents over the tissue scale of centimeters, while at the same time triggering rapid hyperactivity more remotely via synaptic connections. Temporally, we are interested in aberrant spiking activity over milliseconds up to the progression of ischemia extending over a period of hours. We provide our simulation with locations of blood vessels providing a field of oxygen concentration. Disruption at one location produces a central area of cell damage to provide the ischemic core -- the central area where cells are deprived of oxygen and glucose. Toxic agents extending from this core then set up the penumbra, where cells can potentially be saved or protected. In order to cover this vast range of scales, we have developed a mosaic

multiscale model in the NEURON simulator. The notion of a mosaic highlights the contrasting aspects of the model: some components represented in great detail, down to molecular levels, and others that are only represented at the highest level. Into this mosaic we place electrophysiology and intracellular chemophysiology in the context of broad diffusion in the extracellular space.
The development of the mosaic multiscale model thus far includes multiple scales. We explore how tissue-scale ischemic effects produce cellular level changes such as programmed cell death via caspase cascades . Intracellular ischemic cascades also involve calcium induced calcium release. Extracellular space (ECS) simulation provides the ability to test the correlations between edema, astrocyte function, and neuronal outcome.

Disclosures: A. Seidenstein: None. **W.W. Lytton:** None. **R.A. McDougal:** None. **M.L. Hines:** None.

Poster

517. Ischemia: Molecular Mechanisms

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Topic: C.07. Ischemia

Support: NIEHS R01 - ES022936

Title: Mutation and phosphorylation of the apoptosis signal-regulating kinase 1 (ASK1) in oxidative stress signaling

Authors: *A. D. PEGGINS[1], A. PALUBINSKY[2], J. FEDERSPIEL[3], D. LIEBLER[3], B. MCLAUGHLIN[2];
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Abstract: Apoptosis signal regulating kinase 1 (ASK1) is a mitogen-activated protein kinase (MAPK) kinase kinase (MAP3K) that acts as a cellular stress sensor, responding to a multitude of oxidative and energetic events, including reactive oxygen species, tumor necrosis factor alpha stimulation, endoplasmic reticulum stress, increases in intracellular calcium concentration, and reactive lipid electrophiles. Upon challenge by any of these stressors, ASK1 activates MAPK kinases (MAP2Ks) via phosphorylation, leading to even further downstream signaling changes in MAPKs such as p38 and c-JUN N-terminal kinases (JNKs). This signaling cascade ultimately results in activation of the stress response and apoptosis. ASK1 enzymatic activity is regulated by protein-protein interactions. Here we show that ASK1 is part of a dynamic multi-protein

signalosome, the components of which vary based on the type of stress encountered. We show all of the ASK1 interacting proteins detected in ASK complex dynamics, compared to proteins which significantly increased in abundance with increasing HNE concentration. ASK1 is also known to be regulated at the level of post-translational modifications, in which phosphorylation of specific ASK1 residues confers increased or decreased activation. The conditions that result in phosphorylation of specific ASK1 sites remain poorly understood. We have found that different *in vitro* treatments result in activation at varying phosphorylation sites. Regarding differential stress sensing phosphorylation, we have shown that HNE can increase phosphorylation of Serine83 (S83) and Sernie1004 (S1004) where H_2O_2 can decrease phosphorylation of Serine83 (S83) and Sernie1004 (S1004) in a concentration dependent manner. Using these newly identified, treatment-specific sites, ongoing experiments employ CRISPR to investigate the role of such post-translational events in oxidative stress signaling. Future experiments are aimed at further understanding the structural constituents of ASK1 necessary for its role in apoptotic signaling in models of oxidative stress, energetic stress, and ischemia, which encompasses both energetic and oxidative components.

Disclosures: **A.D. Peggins:** None. **A. Palubinsky:** None. **J. Federspiel:** None. **D. Liebler:** A. Employment/Salary (full or part-time): Vanderbilt University. **B. McLaughlin:** A. Employment/Salary (full or part-time): Vanderbilt University.

Poster

517. Ischemia: Molecular Mechanisms

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Title: DREAM\HDAC4\HDAC5 complex downregulates NCX3 expression in Brain Ischemia determing neuronal death

Authors: L. FORMISANO[1], N. GUIDA[2], G. LAUDATI[3], L. MASCOLO[3], M. CANTILE[3], O. CUOMO[3], G. PIGNATARO[3], G. DI RENZO[3], *A. SCORZIELLO[4], L. ANNUNZIATO[4];

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Abstract: The transcriptional mechanism by which stroke induces the reduction of the neuroprotective Na^{+}-Ca^{2+} exchanger isoform 3 (NCX3) is still unclear. Here, we investigated the role of the histone deacetylases (HDACs) in regulating NCX3 in cortical neurons treated with different HDAC inhibitors (HDACi). Trichostatin A, a pan-inhibitor, and MC1568, a class IIA HDACi, significantly increased NCX3 promoter activity, gene and protein expression, whereas MS275, a class I HDACi, had no significant effect. Interestingly, in neurons transiently overexpressing or knocking-down for HDAC4 and HDAC5 NCX3 mRNA and protein levels were down-regulated or increased, respectively. However HDAC7 and 9 had no reliable effect. Furthermore, by Chromatin Immunoprecipitation (ChIP) assay we found that HDAC4 and HDAC5 binding and acetylation of histone protein H3 were reduced or up-regulated after MC1568 treatment. Immunoprecipitations experiments demonstrated also that HDAC4 and HDAC5 are bound to the transcriptional repressor Downstream Regulatory Element Antagonist Modulator (DREAM) and that DREAM knockdown by small interfering RNA (siRNA) transfection prevented HDAC4 and HDAC5 binding to the NCX3 promoter. Moreover, in MC1568 treated neurons site direct mutagenesis of DREAM sequence on ncx3 promoter, corroborated the hypothesis that HDACi-induced NCX3 gene transcription is achieved by DREAM. Furthermore, we found that transient middle cerebral artery occlusion (tMCAO) increased the protein expression of DREAM, HDAC 4 and 5, that in turn, by deacetylating ncx3 gene promoter, determines its down-regulation. Moreover, tMCAO-induced NCX3 reduction was prevented when DREAM, HDAC4 and HDAC5 were silenced by intracerebroventricular (icv) injection of siRNAs for DREAM, HDAC4 and HDAC5. In addition, in neurons silenced with siRNA of DREAM, HDAC4 and HDAC5 and subjected to (OGD) (3 h) plus reoxygenation (RX) (48 h), the neurodetrimental effect of anoxia was counteracted by preventing NCX3 mRNA and protein downregulation. Collectively, our findings suggest DREAM/HDAC4/HDAC5 complex as a new transcriptional repressor machinery that reduces ncx3 transcription in brain after stroke. In addition we identify that the blocking of HDAC4 and HDAC5 could represent a new therapeutic strategy in stroke treatment.

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Poster

517. Ischemia: Molecular Mechanisms

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Support: Adelson Foundation

Burke Medical Research Institute

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Title: A comprehensive analysis of the redox modulation of the adaptive genetic response to hypoxia in neurons: implications for brain protection and repair

Authors: *A. KUMAR[1], M. VAISH[2], A. A. STARKOV[3], I. GAZARYAN[1], S. S. KARUPPAGOUNDER[1], R. R. RATAN[1];
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Abstract: Metazoans have evolved an elegant adaptive program to compensate for hypoxia via a key family of transcription factors, called the hypoxia inducible factors. Under normoxic conditions, the most well studied member of this family, Hif1α is hydroxylated at proline 402 and 564 of its oxygen dependent domain (ODD), leading to its ubiquitination and degradation. Under hypoxic conditions, Hif1α gets stabilized, dimerizes with its partner (Hif-1β) and activates the expression of an array of genes involved in compensating for a discrepancy in oxygen demand and supply. A pervasive but still controversial theory holds that hypoxia activates Hif1α via a mitochondrial peroxide messenger (hydrogen peroxide) that leads to the inhibition of the HIF prolyl hydroxylases, oxygen sensors that directly regulate HIF stability. Other studies have implicated superoxide, lipid peroxides, decrease in oxygen or even decreases in ROS to the hypoxic adaptive response. To reconcile these apparently conflicting observations, we first assessed the response of endogenous antioxidants to hypoxia, as an indirect way to understand the possibility of change in the level of oxidants under hypoxia. Steady state levels of endogenous antioxidants didn't change under hypoxia. Then, in order to understand whether ROS is really involved in hypoxic stabilization of Hif-1α or not, we systematically manipulated the level of ROS in human neuroblastoma cells via adenoviral delivery of a host of antioxidant enzymes including MnSOD, GPX1, GPX4, Catalase and peroxiredoxin 3 (Prdx3) and studied the effect of forced expression on HIF-1 stabilization in normoxia or hypoxia. We examined the effect of these manipulations also on the expression of Hif1α target genes, p21 and enolase 2. Some antioxidants enzymes such as MnSOD (targets Mitochondrial SOD) and GPX4 (targets lipid peroxides) didn't change HIF levels or transcriptional activity. However, GPX1 (targets peroxide) led to a significant decrease in HIF levels and activity while other antioxidants such as Catalase and Prdx3 resulted in a significant increase in HIF levels and activity. A similar trend of change in the stabilization of Hif-1α in mouse primary cortical neurons was confirmed. We conclude that the stabilization of Hif-1α is unrelated to the changes in the level of specific ROS under hypoxia, but that HIF activity can be modulated via specific antioxidant enzymes. The effect of these enzymes is likely redox dependent but experiments are ongoing to establish this connection.

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Poster

517. Ischemia: Molecular Mechanisms

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Topic: C.07. Ischemia

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Title: Rapid and reversible F-actin reorganization as a cytoskeletal hallmark of excitotoxic neuronal cell stress

Authors: B. CALABRESE[1], J. T. AUNG[1], L. HUBER[1], E. R. MORENO[1], R. POWERS[1], Y. ZHANG[1], H. N. HIGGS[2], *S. L. HALPAIN[3,1];
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Abstract: Excess glutamate represents a cellular stress condition that potentially underlies circuit disruption in disorders ranging from acute injury and neurodegeneration to neuropsychiatric disorders. ER and mitochondria fragmentation are two hallmarks of cellular stress in neuronal and non-neuronal cells. Indeed, when we incubate cultured neurons with the glutamate agonist N-methyl-D-aspartate (NMDA) for 5 min, a condition that elicits a form of stress called cytotoxic edema, both organelles undergo extensive fragmentation and/or swelling. We found that bath-applied NMDA also induces a dramatic relocalization of filamentous actin within the somatodendritic domain of a majority of neurons. F-actin is rapidly depleted from dendritic spines and aberrantly aggregates in bundles within the dendrite shaft. Blocking protein synthesis did not prevent this “actinification” of the somatodendritic domain, indicating that pre-existing actin generates these filaments. Either ATP depletion or inhibition of actin polymerization by cytochalasin D (cytoD) prevented the F-actin reorganization, implying that it is an energy-dependent process requiring barbed end actin polymerization, rather than translocation and aggregation of F-actin. This NMDA-induced somatodendritic actin assembly is formin-mediated, not Arp2/3 mediated. We identified inverted formin 2 (INF2) as playing a critical role in this “actinification” of the soma/dendrite, since either shRNA-mediated knockdown or expression of a dominant negative form of INF2 prevented NMDA-induced actinification. Moreover, overexpression of INF2 increased the sensitivity of neurons to actinification. Once induced, somatodendritic F-actin bundles are highly stable, since incubation for several minutes with the G-actin sequestering compound latrunculin A fails to dissipate them,

indicating slow turnover. However, somatodendritic actinification spontaneously reverses once the NMDA receptor activation ceases. Therefore, we propose that this dramatic F-actin reorganization is a pro-survival mechanism, similar to the previously described ER and mitochondria fragmentation, potentially relevant to neurological conditions characterized by excessive depolarization and NMDA receptor hyperactivity.

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Poster

517. Ischemia: Molecular Mechanisms

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NIH RO1 NS081055

The Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Wnt7a in post-stroke angiogenesis: a novel target for stroke repair?

Authors: *H. ZHAO[1], T. B. LENGNING[2], A. J. BRUMM[1], M. MACHNIKI[1], S. T. CARMICHAEL[1];
[1]Neurol., UCLA, Los Angeles, CA; [2]Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Stroke is a leading cause of adult disability, but despite its prevalence current treatments do not provide long-term recovery. Stroke itself induces a wide-range of repair mechanisms, including the proliferation and long-distance migration of immature neurons (neuroblasts) from the subventricular zone (SVZ) to peri-infarct tissue, a process termed post-stroke neurogenesis. These neuroblasts localize to and migrate along angiogenic blood vessels, forming a neurovascular niche in the peri-infarct zone. The reciprocal signaling between neuroblasts and endothelial cells has not been clearly characterized. Understanding these endogenous signaling systems may provide novel targets for pharmaceutical intervention to enhance functional recovery.
In genome-wide expression profiling studies, we compared FACS isolated peri-infarct neuroblasts and neuroblasts from the olfactory bulb. We then identified Wnt7A as a candidate signaling gene that is down-regulated in stroke-responsive neuroblasts. Wnt7A induces endothelial cell migration in vitro and is important in vascular development (Daneman, *et. al*);

however, its role in adult angiogenesis and in stroke repair is not well understood. Based on these findings, Wnt7A may play a key role in the neurovascular niche by promoting neuronal differentiation as well as angiogenesis and vessel maturation. Here, we identify Wnt7A's role in post-stroke angiogenesis in a set of gain-of-function (GOF) and loss-of-function (LOF) studies in the photothrombotic stroke model. Lentiviruses for overexpression and microRNA-mediated knockdown were injected into young adult mice into the peri-infarct cortex, a site for the neurovascular niche that supports post-stroke neurogenesis. At 14 and 42 days after stroke, we found that Wnt7A GOF results in increased vessel density (determined using CD31-positive endothelial cell volume/area). Moreover, CD31/EdU co-labeling indicated an increase in endothelial cell proliferation. We also observed that Wnt7A GOF increases the number of newborn endothelial cells expressing BBB-specific glucose transporter 1 ($GLUT1^{+}/EdU^{+}$) and stimulates overall GLUT1 expression after PT stroke. We are in the process of establishing whether Wnt7A LOF impairs post-stroke endothelial cell proliferation and maturation and whether this would affect overall vessel volume in peri-lesional tissue. Based on our results, overexpression of Wnt7A promotes early angiogenic events (i.e. EC proliferation), enhances the expression of BBB-specific transporters (i.e. GLUT1), and results in morphological changes that persist at least for 7 weeks after stroke (i.e. vessel density).

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Poster

517. Ischemia: Molecular Mechanisms

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Title: Molecular mechanism of hypoxia-induced axon degeneration in cultured cerebellar slices

Authors: *Y. CUI[1], B. G. KIM[2];
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Abstract: Subcortical ischemic vascular dementia is caused by chromic ischemia due to narrowing of small blood vessels supplying the white matter. The pathological changes of ischemic white matter degeneration include axonal degeneration, demyelination, and glial activation. However, it is not fully understood how chronic ischemia leads to the white matter pathologies. In this study we aimed to develop an *in vitro* model of ischemic white matter degeneration using cultured cerebellar slices. Cerebellar slices were obtained from postnatal day 12 mice and cultured for 12 days *in vitro*. At this time point, subcortical white matter axon bundles survived with exuberant myelination. Most of surviving axons from cerebellar cortices were positive with purkinje cell marker calbindin-D28k. The cultured cerebellar slices were exposed to 2% hypoxic condition for 48 hours beginning at 10 day *in vitro*. Hypoxic insult resulted in axons with beading appearances consisting of focal swelling and constriction. In contrast, myelin basic protein expression was preserved even at the site of severe axonal damages. Moreover, there was no significant decrease or loss of oligodendrocytes, suggesting that axonal structures are more vulnerable to hypoxia than myelin ensheathment. Electron micrographs showed accumulation of lysosome-like structures in the axoplasm after hypoxia. Consistent with the EM findings, lysosomal-associated membrane protein 1 (LAMP1) immunereactivity was significantly increased in the white matter in a hypoxic condition. The AMPA/kainate receptor antagonist NBQX, calpain inhibitor ALLN and MDL28170, and lysosomal protease inhibitor pepstatin A, significantly attenuated hypoxia-induced beading appearances. Our slice model could be utilized to explore molecular mechanisms of ischemia-induced axon degeneration. Axonal degeneration following hypoxia is likely to involve multiple pathways, such as AMPA receptor, calpain proteolysis and lysosomal proteases.

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Poster

517. Ischemia: Molecular Mechanisms

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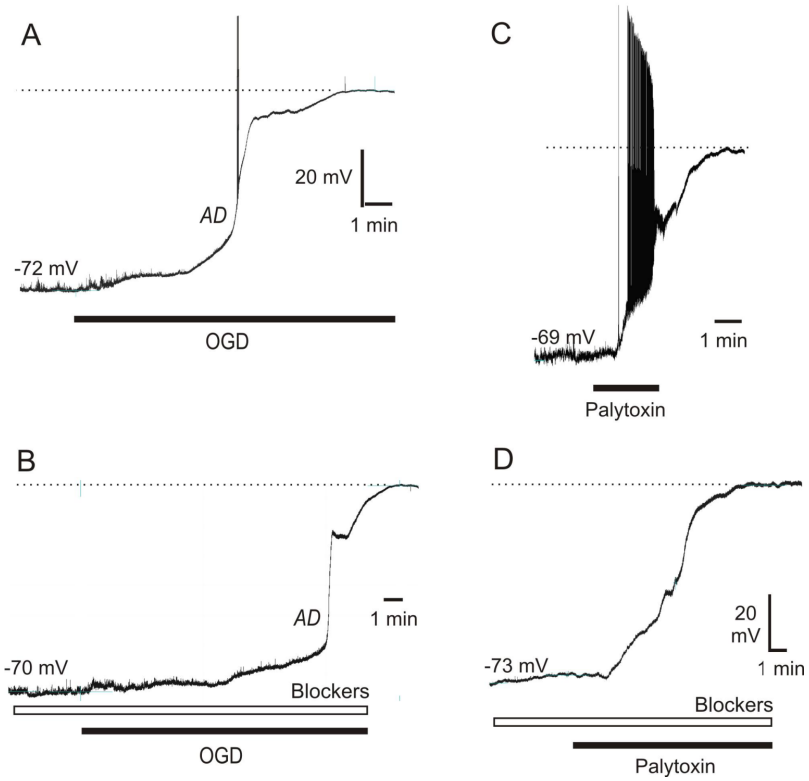
Topic: C.07. Ischemia

Support: Heart & Stroke Foundation of Canada

Title: Still not identified: The channel driving spreading depolarization during ischemia

Authors: *P. J. GAGOLEWICZ, K. TRESIDDER, R. D. ANDREW, K7L 2G8; Queen's Univ., Kingston, ON, Canada

Abstract: Higher CNS neurons undergo a propagating anoxic depolarization (AD) during ischemia that causes acute neuronal injury. There is no consensus as to the origin of the large inward current driving AD. Various scenarios include excess glutamate release or accumulation, open voltage-gated Na^+channels or open pannexin, ASIC, P2X7 or TRPM7 channels. To address this question, we monitored AD in live brain slices during 10 min of O_2/glucose deprivation (OGD) at 35^{o}C using two techniques: 1) AD imaged as a wave of increased light transmittance (LT) propagating across neocortex; 2) sudden AD onset in whole-cell patch recordings from neocortical pyramidal neurons. LT imaging showed an intact propagating AD front despite slice pre-treatment with one of 2 mixtures of blockers in artificial CSF. Blocker `mix 1` contained: 1 uM TTX, 10 mM TEA, 1 mM kynurenic acid, 10 uM nifedipine, 100 uM carbenoxolone/500 uM probenicide [pannexin blockers] and 100 uM picrotoxin. Blocker `mix 2` contained: 100 uM amiloride [ASICs], 2 uM BBG [P2X7], 2 uM FTY720 [TRPM7] and 100 uM CSB [glutamate uptake]. Whole-cell patch recordings from pyramidal neurons showed that OGD induced AD within 5.5 to 8.5 min (n=10, panel A). Pre-treatment with `mix 1` merely delayed AD onset (9.2 to 12 min, n=8, panel B). Mix 2 had no effect on onset (6 to 8 min, n=7). We conclude that the usual channel suspects do not drive AD. Is there an alternative? Like OGD, 10-100 nM palytoxin evokes a spreading AD-like event as imaged in 12 slices. An abrupt depolarization evoked by 50-100 nM palytoxin in pyramidal cell recordings (n=7, panel C) was only delayed by pre-treatment with mix 1 (panel D) similar to OGD. Mix 2 was without effect (n=7). Because palytoxin converts the Na/K pump into an *open cationic channel*, ischemia might elicit a similar pump conversion that drives AD and subsequent acute neuronal damage.



Disclosures: **P.J. Gagolewicz:** None. **K. Tresidder:** None. **R.D. Andrew:** None.

Poster

517. Ischemia: Molecular Mechanisms

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Support: CSIR India UNDO-BSC0103

Title: Role of nuclear class III histone deacetylases in differentially affected brain areas following transient global cerebral ischemia

Authors: A. B. WAHUL[1], P. JHELUM[1], R. KORABOINA[1], *A. KUMAR[2], S. CHAKRAVARTY[1];
[1]Dept. of Chem. Biol., Indian Inst. of Chem. Technol., Hyderabad, India; [2]CSIR- Ctr. For Cell. and Mol. Biol. (CCMB), Hyderabad, India

Abstract: Transient global cerebral ischemia is the result of cardiac arrest, which finally leads to broad range of neurological and emotional dysfunction, including cognitive ones. To understand the details of the molecular events underlying ischemia-induced damage and repair, we performed bilateral common carotid arterial occlusion (BCCAo) in adult CD1 male mice for 6-7 minutes, followed by reperfusion. Animals were sacrificed at 24, 72 and 120 h post ischemia and reperfusion, to uncover the molecular mechanisms involved in damage as well as neurogenesis that ensues. After ischemic insult, each animal was assessed for motor coordination as well as grip strength functionality. Visual memory was also analyzed using novel object recognition (NOR) test. To evaluate the degree of damage in three different affected brain areas in mice subjected to BCCAo, micro-dissected samples from cortex, striatum and hippocampus were processed for molecular as well as histochemical investigations. The neurobehavior assessment showed loss of motor co-ordination as well as grip strength functionality at early time point, which slowly recovered over time 72 h, but the visual memory functionality remained affected even 72 hours post-ischemic insult. Further, there was significant loss of synaptic connectivity at early time point, as confirmed by the Western blot analysis. For molecular mechanisms the genes investigated were (i) Hypoxia Inducing factors (HIF 1 &2), hypoxia markers (ii) Vascular Endothelial Growth Factor (VEGF), angiogenic markers, (iii) Neuroglial damage markers (Aqp4, neurabin2, GFAP), (iv) inflammatory markers (IL1α and IL6). Finally, we studied the epigenetic regulation of few of these genes by NAD-dependent class III HDACs called Sirtuins, in particular the nuclear ones i.e. Sirt1, 2, 6 and 7, in the etiopathology as well as recovery. The outcome of the study yielded better insight into the molecular mechanisms underlying neural damage and recovery following BCCAo.

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Poster

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NS089534

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NSFC81271276

Title: Endogenous lipid electrophiles mediate ischemic tolerance in brain via Nrf2

Authors: T. YANG[1], Y. SUN[1], L. MAO[2], B. SUN[2], Y. GAO[1,3], S. H. GRAHAM[1], J. CHEN[1,3], *F. ZHANG[1];
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Abstract: Introduction: Ischemic stroke is the leading cause of long-term disability and third-leading cause of death. Though FDA approved tPA for restoring the blood flow, no medicine is available for the purpose of neuroprotection. Ischemic preconditioning (IPC) is a promising approach for protecting against ischemic injury; however, the underlying protective mechanisms are not clear. Recent studies suggested that nuclear factor erythroid 2 related factor 2 (Nrf2) may underlie the protective mechanism of IPC. This study aims at confirming the role of Nrf2 in IPC and investigating how Nrf2 is activated following IPC. Methods: In vivo, middle cerebral artery occlusion (MCAO) was induced in male C57BL/6J wild type (WT) and Nrf2 knockout mice, 12 min for IPC and 60 min for stroke with an interval of 3 days. In vitro, oxygen-glucose deprivation was applied to rat primary neuronal cultures, 12 min for preconditioning and 60 min for ischemic condition with an interval of one day. Results: In mice, IPC significantly reduced neurological dysfunction and infarct volumes compared to control group. The protection depended on Nrf2, as IPC induced Nrf2 nuclear translocation and upregulation of its target genes on PCR arrays, and Nrf2 knockout abolished the protection of IPC. Similar results were observed in vitro, confirming the key role of Nrf2 in IPC. To identify the endogenous inducers of Nrf2, we performed Western dot blots to measure the levels of 4-hydroxy-nonenal (4-HNE), a lipid electrophiles and the end-product of lipid peroxidation of omega-6 polyunsaturated fatty acids. We found that IPC increased the level of 4-HNE in the brain via mild oxidative stress. In exploring the role of 4-HNE in IPC, we found that 4-HNE alone activated Nrf2 in neuronal

cultures and in brain tissue, and increased the expression of heme oxygenase 1 (HO-1), a target enzyme of Nrf2. In addition, IPC-mediated protection was aborted by the treatment of N-acetylcysteine (NAC), a compound that can neutralizes electrophiles. Taken together, our data suggest that lipid electrophiles induced by IPC could exhibit an endogenous neuroprotective effects against stroke via the activation of Nrf2 pathway. Conclusion: Our findings suggested a novel mechanism of ischemic tolerance, in which lipid electrophiles act as key mediators. IPC induced mild oxidative stress in the brain, leading to the generation of lipid electrophiles; these electrophiles then activate Nrf2 pathway, protecting the brain against stroke.

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Poster

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Topic: C.07. Ischemia

Support: NIH Grant NS067078

Title: Activation of the phb-oma1-opa1 pathway is an early mitochondrial event in neuronal ischemia

Authors: *C. J. ANDERSON, P. ZHOU, G. MANFREDI, C. IADECOLA;
Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstract: Mitochondrial structure is critical for neuronal health. Abnormal mitochondrial structure has been reported in ischemic brain injury and alterations in key mitochondrial structure regulators have been identified. However, the molecular mechanisms that lead to these alterations in ischemia remain to be elucidated. Therefore, we sought to gain insight into molecular determinants of mitochondrial structural changes in ischemic injury. Prohibitin (PHB), an inner mitochondrial membrane (IMM) protein that forms large heteromeric complexes, is essential for mitochondrial structure (PLoS Genet., 8:11, 2012). Previously, we demonstrated that overexpression of PHB protects neurons from ischemic cell death (Stroke, 45:4, 2014) and loss of PHB increases neuronal susceptibility to injury (J. Neurosci., 32:583, 2012). PHB regulates levels of membrane-bound isoforms of OPA1, an IMM protein required for mitochondrial fusion and cristae structure, by preventing its cleavage by the metalloprotease OMA1. We observed that mitochondria become fragmented in primary cortical neurons after 2hrs oxygen-glucose deprivation (OGD), resulting in a 40% reduction of average length, which

persisted throughout OGD (4hrs). Using native gel electrophoresis, we observed that the PHB complex is destabilized by OGD, seen as a reduction in PHB complex size. Consistent with the hypothesis that a role of PHB is to stabilize OPA1, we observed an increase in OPA1 processing of long isoforms 2hrs after OGD, concomitant with mitochondrial fragmentation. Furthermore, we observed an increase in OMA1 activation using an OMA1-FLAG construct. To directly demonstrate the role of OMA1-dependent OPA1 processing in OGD-induced neuronal cell death, we created an OPA1 construct lacking the S1 processing site responsible for cleavage by OMA1 in low ΔΨm conditions (OPA1-ΔS1). Neurons expressing OPA1-ΔS1 were significantly protected from OGD-induced death compared to vector expressing neurons. Interestingly, OPA1-ΔS1 expression did not mitigate mitochondrial fragmentation in OGD. This suggests that in OGD, OPA1-ΔS1 does not protect neurons by maintaining mitochondrial network morphology. In order to understand how PHB controls this pathway we studied the interaction of PHB and OPA1 in N2a cells. Upon treatment with the ΔΨm-dissipating protonophore CCCP, the interaction of PHB and OPA1 decreased, which may indicate a role for PHB in the regulation of OPA1 processing by physical interaction. Together, these data suggest that the PHB-OMA1-OPA1 pathway participates in early mitochondrial damage in OGD, possibly by regulating IMM structure independently of fission and fusion dynamics.

Disclosures: **C.J. Anderson:** None. **P. Zhou:** None. **G. Manfredi:** None. **C. Iadecola:** None.

Poster

517. Ischemia: Molecular Mechanisms

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Title: Absence of Nrf2 preferentially aggravates the early-stage of reactive astrogliosis contributing to the inflammation associated cerebral ischemic damage under hypoxia

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Abstract: Protracted neuroinflammation has been recognized as a pivotal mechanism of secondary brain injury progression following ischemic stroke. Astrocytes are emerging as crucial

regulators of CNS inflammatory response that exert either powerful proinflammatory potential or potent protective anti-inflammatory function. Accumulated evidence indicates that the transcriptional factor Nrf2 and antioxidant response element (ARE) pathways play a vital role in the cellular defense against oxidative stress, inflammation, and cell death via upregulating cytoprotective genes encoding for phase II defense enzymes and antioxidant stress proteins. However, the underlying regulatory mechanism of the Nrf2 pathway in the CNS remains inconclusive. Interestingly, Nrf2/ARE-regulated genes are preferentially activated in astrocytes, thereby having more efficient cytoprotection defenses than neurons. Here, through testing in a hypoxic/ischemic (H/I) mice model, we show that Nrf2 can function as a modulator of reactive astrogliosis that links inflammation in the early stage of ischemic stroke to subsequent brain injury. We found that at 24h after H/I, cerebral infarct size, edema volume, and the resultant functional neurological deficit were significantly increased in Nrf2 knockout mice compared to wild-type controls. To further determine which cells appear to be the most preferentially activated in a spatiotemporal pattern by this Nrf2/ARE pathway contributing to ischemic injury, we investigated, in the very early stage of ischemic injury following H/I, the characteristic changes in brain cells, including neuron loss and degeneration, as well as reactive astrogliosis and microglia/macrophage activation. 12h after H/I, as we expected, the mice lacking Nrf2 exhibited much more severe neuronal death, and more remarkable reactive astrogliosis and microglia activation. Notably, 6h after H/I, Nrf2 ablation dramatically triggered obvious reactive astrogliosis and microglia activation in the striatum. However, neuronal loss did not show an obvious difference between groups through either Cresyl violet or NeuN staining, whereas lack of Nrf2 resulted in relatively high synaptic damage compared to wild-type controls, which might lead to the functional deficit. Indeed, the functional evaluation showed an obviously severe neurological deficit in Nrf2 knockout mice 6h after H/I. Our findings indicate a neuroprotective role of Nrf2 pathway against hypoxia-associated brain diseases, and the Nrf2-dependent cytoprotective response occurs preferentially in astrocytes, providing new insight into the unique pathways involved in the CNS and neurological disorders.

Disclosures: **L. Liu:** None. **M. Vollmer:** None. **M. Carson:** None. **S. Dore:** None.

Poster

517. Ischemia: Molecular Mechanisms

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Topic: C.07. Ischemia

Support: NIH R21NS093522

AHA 13SDG1397009

Title: Human acid-sensing ion channel 1a (ASIC1a) exhibits higher levels of surface trafficking than mouse ASIC1a

Authors: *Y. XU[1,3], Y.-Q. JIANG[2,4], J. WU[2,5], M. HE[2], Y. HU[5], J. XU[3], W. G. RUSYNIAK[6], M. LIN[2], X.-M. ZHA[2];
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Abstract: ASICs are a family of proton-gated cation channels which are mainly expressed in the nervous system. In the brain, the main ASICs contributing to acid-activated currents are ASIC1a, ASIC2a, and ASIC2b. ASIC1a is more pH sensitive and starts to be activated at pH≤7.2, In contrast, ASIC2 mainly plays a modulatory role. ASICs are important for synaptic function, and are one main contributor to acidosis-related neuronal injury. Previous ASIC studies were mainly performed in rodents, and we know little about ASICs in humans. To facilitate the translational targeting of ASICs, it is important to understand the differences between mouse and human ASICs. In our previous studies, we have found that human ASIC1a and mouse ASIC1a exhibit differences in current and their effect on spine remodeling. These data indicate that the two homologs, though sharing 98% amino acid identity, differ in their contribution to acidosis-induced responses. The objective of this study is to determine 1) the molecular mechanism underlying differential function of human and mouse ASIC1a, and 2) their contribution to acidosis-induced neuronal injury. We compared acutely dissected human and mouse brain tissue, and found that human brain expressed lower levels of ASIC2b. Further, since ASIC1a is the key subunit determining acid-activated currents, we focused on ASIC1a and studied the trafficking of the two homologs in heterologous cells. Human ASIC1a exhibited higher surface trafficking, which correlated with an increase in *N*-glycosylation. Inhibiting *N*-glycosylation with tunicamycin reduced surface trafficking of human and mouse ASIC1a to a similar level. Previous studies showed that the interaction between the wrist region and the first transmembrane domain is important for efficient glycosylation and trafficking of ASIC1a. We examined the amino acid sequence of the homologs and found that two amino acids within these two regions, amino acids 70 (His in human; Cys in mouse) and 285 (Pro in human; Ser in mouse), differ between human and mouse ASIC1a. To test whether these two amino acids determine differential trafficking of the two ASIC1a homologs, we generated swapping mutants of human and mouse ASIC1a. Swapping amino acid 70 had little effect. In contrast, the mouse S285P mutant exhibited increased *N*-glycosylation, elevated surface level, and higher acid-activated current. The human P285S mutant had the opposed effect. These results reveal the molecular basis for elevated trafficking and activity of human ASIC1a, and further indicate that acidosis has a bigger impact on neuronal injury in human neurons.

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Poster

517. Ischemia: Molecular Mechanisms

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Suzuken Memorial Foundation (15-075)

Title: Suppressive effect of orexin-A through vagus nerve on the development of post-ischemic glucose intolerance and increase of inflammatory factors

Authors: *S. HARADA, Y. NOZAKI, S. TOKUYAMA;
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Abstract: We have previously found that the development of post-ischemic glucose intolerance is one of the triggers of ischemic neuronal damage. Orexin-A (a neuropeptide in the hypothalamus) plays an important role in many physiological functions, including the regulation of glucose metabolism. We have reported that the vagus nerve plays an important role in the recovery of post-ischemic glucose intolerance and mediates a neuroprotective effect by hypothalamic orexin-A. In addition, orexin neuron projects from hypothalamus to medulla oblongata, nuclei originis of vagus nerve. Recently, it was reported that cerebral ischemia can activate hepatic inflammatory factors and is associated with the development of hepatic insulin resistance by activation of sympathetic nerve. The aim of this study was to determine the involvement of orexin-A and vagus nerve on the cerebral ischemia-induced inflammatory factors in liver.

Male ddY mice were subjected to middle cerebral artery occlusion (MCAO) for 2 h. The hepatic vagotomy mice created to selectively transect at the hepatic branch vagus nerve. Neuronal damage was estimated by histological and behavioral analyses. Expression of each protein levels was analyzed by western blot and immunofluorescence staining.

Intrahypothalamic orexin-A (5 pmol/mouse) administration significantly suppressed the development of post-ischemic glucose intolerance and neuronal damage on day 1 and 3, respectively after MCAO. MCAO-induced decrease of hepatic insulin receptors and increase of hepatic gluconeogenic enzymes on day 1 after was reversed to control levels by orexin-A. This effect was reversed by hepatic vagotomy. In the liver, MCAO-induced increase in F4/80 (a kupper cells marker), TNF-α and IL-1β on day 1 was recovered to control levels by OXA, and which was reversed by hepatic vagotomy.

These results suggest that the orexin-A and vagus nerve may play an important role in the regulation of post-ischemic elevated hepatic inflammatory factors.

Disclosures: **S. Harada:** None. **Y. Nozaki:** None. **S. Tokuyama:** None.

Poster

517. Ischemia: Molecular Mechanisms

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Topic: C.07. Ischemia

Support: NSFC Grant 31171029

Title: Following oxygen-glucose deprivation/reperfusion, nuclear translocation of annexin 1 induces neuronal apoptosis by regulating Bid expression via p53 binding

Authors: ***X. LI**, Y. ZHAO, Q. XIA, L. ZHENG, L. LIU, B. ZHAO, J. SHI;
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Abstract: As a Ca^{2+}-and phospholipid-binding protein, annexin 1 (ANXA1) performs different roles depending on its subcellular localization. Our previous data have suggested that the nuclear translocation of ANXA1 is involved in neuronal apoptosis after ischemic stroke. As the mechanism and function of ANXA1 nuclear migration remain unclear, it is important to clarify how ANXA1 performs its role as an apoptosis "regulator" in the nucleus. Here, we report that importazole (IPZ), an importin β-specific inhibitor, decreased ANXA1 nuclear accumulation and reduced the rate of neuronal death induced by nuclear ANXA1 migration after oxygen-glucose deprivation/reoxygenation (OGD/R). Notably, ANXA1 interacted with the *Bid* promoter directly; however; this interaction could be partially blocked by the p53 inhibitor pifithrin (PFT)-α. Accordingly, ANXA1 was shown to interact with p53 in the nucleus and this interaction was enhanced following OGD/R. A luciferase reporter assay revealed that ANXA1 was involved in the regulation of p53-mediated transcriptional activation after OGD/R. Consistent with this finding, the nuclear translocation of ANXA1 after OGD/R up-regulated the expression of Bid, which was impeded by IPZ, *ANXA1* shRNA, or PFT-α. Finally, cell-survival testing demonstrated that silencing ANXA1 could improve the rate of cell survival and decrease the expression of both cleaved caspase-3 and cleaved poly ADP-ribose polymerase. These data suggested that importin β-dependent nuclear ANXA1 migration participates in the OGD/R-dependent induction of neuronal apoptosis. ANXA1 interacts with p53 and promotes p53 transcriptional activity, which in turn regulates Bid expression. Silencing ANXA1 decreases the expression of Bid and suppresses caspase-3 pathway activation, thus improving cell survival after OGD/R. Overall, these observations provide a more comprehensive understanding of ANXA1 function in cell apoptosis after OGD/R, suggesting the potential for a previously unidentified treatment strategy in minimizing apoptosis after ischemic stroke.

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Poster

517. Ischemia: Molecular Mechanisms

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Topic: C.07. Ischemia

Title: Cerebral ischemia-mediated phosphorylation of GSK-3〈β〉 induced neurogenesis in rats

Authors: ***K. KISOH**, H. HAYASHI, M. ASADA, T. ITO, M. ARAI, B. YUAN, N. TAKAGI; Applied Biochem., Tokyo Univ. Pharm. and Life Sci., Tokyo, Japan

Abstract: Cerebral ischemia is one of the leading causes of death and disability. Therapy against cerebral ischemia is limited. In particular, the therapeutic agents for the treatment during chronic stage of cerebral ischemia are used to prevent of recurrence of stroke. Neurogenesis is expected as new therapeutic approach currently, because it is focused on regenerating damaged neural circuits by development of stem cell research. It is transiently enhanced as one of endogenous regenerative abilities in the subventricular zone (SVZ) of lateral ventricle and the subgranular zone (SGZ) of hippocampal dentate gyrus (DG) in the damaged brain after cerebral ischemia. However, mechanisms of neurogenesis after cerebral ischemia remain to be fully clarified. The aim of this study is to elucidate the mechanism of neurogenesis after cerebral ischemia. Cerebral ischemia was produced by the injection of 700 microspheres into right internal carotid artery of the rat in this study. At first, we examined cell proliferation and differentiation in dentate gyrus (DG) of the hippocampus on day 7 after microsphere-induced cerebral embolism (ME). Ki67-positive proliferated cells in ipsilateral DG were increased compared to those in contralateral DG. These cells also expressed DCX, which is an immature neural marker. In addition, NeuroD, proneural bHLH transcription factor, was expressed in cells in granule cell layer of DG and the number of NeuroD-expressed cells in ipsilateral DG after ME was increased compared to that of contralateral DG on day 7 after ME. We next demonstrated that Akt/GSK-3〈β〉/〈β〉-catenin signaling, which is involved in neurogenesis and regulate expression of NeuroD, was promoted on day 7 after ME. Our present data suggested that NeuroD expression on day 7 after ME might have been caused by Akt/GSK-3〈β〉/〈β〉-catenin signaling pathway, including increase in phosphorylation of GSK-3〈β〉. As indicated changes of Akt/GSK-3〈β〉/〈β〉-catenin signaling pathway, we further examined that the levels of IGF-1 and BDNF are involved in this process, which are closely related to neurogenesis via PI3K/Akt patheway. Contrary to expectations, levels of these growth factors were significantly decreased on day 7 after ME.

These results suggest that ME-induced cell proliferation and differentiation are involved in Akt/GSK-3〈β〉/〈β〉-catenin signaling in IGF-1 and BDNF-independent manners at this time point.

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Poster

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Topic: C.07. Ischemia

Support: NIH Grant NS95192

Title: Dna methylation and hydroxymethylation patterns in mouse brain following experimental stroke.

Authors: *K. MORRIS, R. VEMUGANTI;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: Mounting evidence over the past decade reveals that epigenetic factors play a major role in the pathophysiology of ischemic injury in the brain. Cerebral ischemia has been shown to alter the methylation and hydroxymethylation of DNA which are interrelated epigenetic processes associated with the repression or activation of transcription, respectively. Inhibition of DNA methyltransferases provides robust protection against ischemic injury. Alternatively, inhibition of a Ten-eleven translocation (TET) dioxygenase, an enzyme that converts methylated DNA to hydroxymethylated DNA was shown to increase infarct volume following transient middle cerebral artery occlusion (MCAO) in adult rodents. We presently determined the effect of transient MCAO on DNA methylation and hydroxymethylation in mouse brain. C57BL/6J male mice were subjected to 60 min of MCAO and the cortex, striatum, and hippocampus were harvested at 5 min, 3h, 6h and 24h of reperfusion. Dot blot analyses of DNA showed significant increases in DNA methylation at 5 min, 3h and 6 h of reperfusion in the cortex, and at 6h of reperfusion in the hippocampus. DNA hydroxymethylation was also increased in the cortex at 5 min of reperfusion, whereas remained unaltered in the hippocampus and striatum. We also assessed the expression of the DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b) and the methyl dioxygenases (TET1, TET2, and TET3). Following transient MCAO, DNMT3a and DNMT3b were significantly increased in both cortex and striatum. Whereas, TET1 was increased in the cortex while TET3 was increased in the striatum. Overall these data indicate the

changing temporal landscape of DNA methylation and hydroxymethylation following cerebral ischemia. These results may be important for enhancing the efficacy of potential stroke therapeutic targets that effect DNA methylation. Funded by NIH.

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Poster

517. Ischemia: Molecular Mechanisms

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Topic: C.07. Ischemia

Support: DFG Grant KA 3810/1-1

Title: Mitochondrial Complex I-mediated bioenergetic failure in acute ischemic brain injury

Authors: *A. KAHL[1], A. STEPANOVA[2], C. KONRAD[1], G. MANFREDI[1], P. ZHOU[1], A. GALKIN[2], C. IADECOLA[1];
[1]Feil Family Brain and Mind Res. Inst., Weill Cornell Med., New York, NY; [2]Sch. of Biol. Sci., Queen's Univ. Belfast, Belfast, United Kingdom

Abstract: Mitochondrial energy production in the brain fails rapidly at the onset of ischemia and is only restored by timely reperfusion. The loss of cerebral blood flow leads to decreased oxygen levels and energy crisis in the ischemic area, initiating a sequence of pathophysiological events that after reoxygenation lead to ischemia/reperfusion (IR) damage (Neuron 67:181, 2010). Mitochondria play a key role in ischemic brain injury. Oxidative stress and bioenergetic failure have been proposed as early events in IR damage (Cell Metab. 23:254, 2016). However, the biochemical and molecular mechanisms of mitochondria damage in IR remain to be elucidated. We studied IR-induced changes of mitochondrial respiratory function in a mouse model of focal ischemia induced by transient occlusion of the middle cerebral artery (MCA). Reestablishing cerebral blood flow after MCA occlusion resulted in a significant decline (50±6.2% of sham control, mean±SEM; $p<0.05$; n=5/group) in ADP-stimulated respiration measured in tissue homogenates from the ischemic area. The decline was followed by a recovery of mitochondrial oxygen consumption at one hour after reperfusion (85±2.3% of sham control; $p>0.05$; n=5). After the transient restoration in mitochondrial function, a secondary gradual decline in respiration occurred during the following 4-24 hours (55±7.8% of sham control; $p<0.05$; n=5, at 24h). Mitochondrial respiratory control ratio (phosphsorylating:non-phosphorylating respiration), a parameter reflecting the integrity of the mitochondrial membrane, did not change during the first 12 hours of reperfusion, suggesting that opening of the permeability transition pore was

unlikely to be the underlying cause for the respiratory decline in this time period. Functional analysis of individual respiratory chain complexes indicated that reduced mitochondrial respiration was associated with a decline in mitochondrial complex I activity. *In vitro* treatment of post-ischemic mitochondrial fractions with a thiol reducing agent partially recovered complex I activity at early, but not at late, time points after reperfusion. These data suggest that early reversible post-translational modifications of complex I were followed by irreversible enzyme deficiency. Our results suggest a central role of complex I impairment in the bioenergetic failure initiating ischemic brain injury. Modulating complex I-dependent bioenergetic failure could be an effective approach to prevent subsequent detrimental events in the IR cascade, ultimately reducing brain damage after stroke.

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Poster

517. Ischemia: Molecular Mechanisms

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Title: The role of developmental transcription factors in post-stroke axonal sprouting

Authors: *C. A. SCHWEPPE, M. MACHNICKI, S. T. CARMICHAEL;
Univ. of California Los Angeles, Los Angeles, CA

Abstract: The ability of the nervous system to repair itself following injury is a remarkable phenomenon, but central nervous system endogenous repair mechanisms are incomplete. Ischemic stroke produces a limited process of neural repair, characterized by axonal sprouting and cortical reorganization in the cortex adjacent to the infarct (peri-infarct region). Previously, our lab has used transcriptional profiling of single sprouting neurons during the period of post-stroke axonal sprouting to identify their unique gene expression profile, a post-stroke sprouting neuron transcriptome. Within this transcriptome dataset are a number of transcription factors known to play a role in cortical development that are differentially regulated in sprouting neurons following stroke. Developmental transcription factors present an intriguing target for study due to their inherent ability to act as master regulators of genes responsible for neuronal growth. As such, a number of these differentially regulated developmental transcription factors have been investigated for their ability to regulate axonal sprouting following stroke.

Transcription factor targets were initially screened for their ability to promote axonal outgrowth following lentiviral overexpression and knockdown in mouse primary cortical neuron cultures. To test the ability of these targets to promote post-stroke axonal sprouting in vivo, adult C57BL/6 mice received a cortical stroke via middle cerebral artery occlusion followed by viral delivery of expression vectors. Relative axonal sprouting was evaluated via BDA tracing of connections in the peri-infarct region. The results of these studies begin to explore the role of development-associated transcription factors in neural regenerative processes in the adult cerebral cortex.

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Poster

517. Ischemia: Molecular Mechanisms

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Support: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-0002316)

Title: Cerebroprotection by hesperetin against ischemic brain injury via restoration of tryptophan-modulating enzymes

Authors: *W. LEE, S. KIM, C. KIM;
Pusan Natl. Univ. Sch. of Med., Yangsan, Gyeongsangnam-Do, Korea, Republic of

Abstract: Indoleamine 2,3-dioxygenase (IDO) and tryptophanyl-tRNA synthetase (TrpRS) are tryptophan-modulating enzymes involved in L-tryptophan catabolism and its use in protein synthesis, respectively, and are associated with the neuroinflammation and immune response to acute cerebral ischemia. Hesperetin, a citrus flavanone, is known to help prevent tissue damage from oxidative stress in the brain. In this study we investigated the mechanisms involved in cerebroprotective action of heseperetin in connection with the tryptophan-modulating enzymes. Male C57BL/6 mice were anesthetized and subjected to photothrombotic cortical ischemia. Hesperetin was administered i.p. 1 h after ischemic insult. Posttreatment with hesperetin significantly reduced the infarct size including infarct area and volume. Ischemic insult markedly altered the tryptophan-modulating enzymes, i.e. an increase in the expression of IDO and a simultaneous decrease in that of TrpRS. Hesperetin significantly decreased the expressions of IDO, CD11b, CD11c, p-JAK2 and p-STAT1 via increasing the expression of TrpRS. Hesperetin significantly restored the tryptophan-modulating enzymes, inhibiting JAK/STAT signaling

activation. These results suggest that the cerebroprotective effects of hesperetin might be associated with the increase in the TrpRS expression as well as the suppression of the IDO expression.

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Poster

517. Ischemia: Molecular Mechanisms

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RO1 NS080177

Title: mir-29a differentially regulates cell survival in astrocytes from cornu ammonis-1 and dentate gyrus by targeting VDAC1

Authors: ***C. STARY**[1], X. SUN[1], Y. OUYANG[1], L. LI[1,2], R. GIFFARD[1];
[1]Anesthesia, Stanford Univ., Stanford, CA; [2]Anesthesia, Zhujiang Hospital, Southern Med. Univ., Guangzhou, China

Abstract: Neurons in the cornu ammonis 1 (CA1) region of the hippocampus are selectively vulnerable to transient cerebral ischemia, while neurons in the dentate gyrus (DG) are more resistant. This effect is mediated by local astrocyte function, and may be related to differences in subregional hippocampal expression of miR-29a. In the present study we investigated the role of miR-29a on cell survival in hippocampal astrocytes cultured selectively from CA1 and DG in response to extended glucose deprivation (GD) injury. We observed that CA1 astrocytes exhibited a higher degree of cell death and a correspondingly greater decrease in miR-29a following injury versus astrocytes from DG. A reciprocal change was observed in expression of the mitochondrial voltage dependent cation channel-1 (VDAC1), a regulator of mitochondria and target of miR-29a. In both CA1 and DG astrocytes, increasing levels of miR-29a by transfection with mimic decreased VDAC1 expression and improved cell survival following injury. Knockdown of VDAC1 expression with small interfering RNA resulted in improved survival from GD injury in both CA1 and DG astrocytes. Finally, we observed that the protective effect

of miR-29a was eliminated by inhibition of miR-29a/VDAC1 binding. These findings suggest that the selective vulnerability of the CA1 to transient cerebral ischemia may be due in part to a limited miR-29a response in CA1 astrocytes. This effect results in a relative increase in VDAC1-mediated cellular dysfunction in CA1 astrocytes. These findings may provide insight for post-resuscitation interventions aimed at preventing hippocampal injury following global cerebral ischemia.

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Poster

517. Ischemia: Molecular Mechanisms

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Title: Ischemia/reperfusion injury leads to accumulation of insoluble protein aggregates with components similar to neurodegenerative diseases

Authors: *K. HOCHRAINER[1], A. KAHL[1], K. JACKMAN[1], J. BASKAR[1], S. ZHANG[2], J. ANRATHER[1], C. IADECOLA[1];
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Abstract: Brain injury is frequently associated with the accumulation of ubiquitin-containing insoluble protein aggregates (Ross and Poirier, 2004). We have recently discovered that cerebral ischemia leads to a drastic increase in ubiquitin-rich inclusions analogous to other neurological disorders (Hochrainer et al, 2012; Hochrainer et al, 2015). To gain a better understanding of the biological significance of post-ischemic aggregates we asked what other proteins in addition to ubiquitin migrate to protein aggregates after stroke. Mice underwent middle cerebral artery occlusion or sham-surgery (n=8/ group). After one-hour reperfusion, when ubiquitin is maximally detected in aggregates, mice were sacrificed and insoluble proteins, as determined by their resistance to 2% Triton X-100 solubilization, were obtained from ischemic neocortices. Isolated proteins were digested with trypsin and identified by nanoLC-MS/MS (n=4, each 2 pooled sham and ischemia animals). Relative amounts of proteins detected in sham and ischemia

groups were quantified for each run using label-free quantification (MaxQuant). Ischemia versus sham fold change was determined and mean values were calculated across all 4 runs. Out of 541 identified proteins, 197 were significantly increased in the insoluble aggregate fraction after ischemia/reperfusion (fold change 1.21 - $6.24x10^7$). As anticipated, ubiquitin was among the most prevalent proteins ($5.83x10^7 \pm 2.91x10^7$-fold induction). Stroke-induced insoluble proteins were examined for Gene Ontology enrichment by the DAVID database (https://david.ncifcrf.gov). Ischemia/reperfusion almost exclusively promoted the aggregation of proteins regulating RNA/protein synthesis and signal transduction ($P<0.001$), while it either reduced or did not affect insoluble proteins associated with membranes, cytoskeleton and macromolecular complexes. Finally, stroke-dependent aggregated proteins were analyzed for functional association networks via the STRING database (http://string-db.org) and the 2 largest clusters were examined for identity of proteins. Remarkably, we found in these clusters a notable number of RNA-binding proteins with known relation to neurodegenerative diseases, especially ALS and FTD, among them Tdp43, Fus, Hnrnpa1, Hnrnpa3, Sfpq and Srsf1. Our data support a novel and previously unrecognized molecular overlap between neurodegeneration and ischemic stroke. Although aggregation of these proteins is believed to be detrimental in neurodegenerative diseases, it remains to be established how the accumulation of these proteins affects cell viability and outcome after cerebral ischemia/reperfusion.

Disclosures: **K. Hochrainer:** None. **A. Kahl:** None. **K. Jackman:** None. **J. Baskar:** None. **S. Zhang:** None. **J. Anrather:** None. **C. Iadecola:** None.

Poster

517. Ischemia: Molecular Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 517.25/FF1

Topic: C.07. Ischemia

Support: National Natural Science Foundation of China grant (81571125)

Title: Myeloid differentiation primary response protein 88 protects cerebral ischemia via a non-inflammatory mechanism

Authors: *J. REN, Y. HE, K. QIAN, Y. SHEN, Y.-D. ZHOU;
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Abstract: Toll-like receptors (TLR) are a group of pattern recognition receptors which play a critical role in the innate immune system. Myeloid differentiation primary response protein 88 (MyD88) is one of the key molecules recruited by the toll-like receptors. Existing evidence

shows that TLR/MyD88-mediated inflammatory processes within the brain might contribute to brain ischemic injury. However, knocking out MyD88 aggravates brain ischemic injury. In the present study, we explored the mechanism underlying MyD88-mediated protection against brain ischemia. We found that MyD88 expression level was lower in hippocampal CA1 area than in CA3 and cortical regions in C57BL/6 mice. The expression level of MyD88 correlates well to its protection against transient forebrain ischemia-induced neurodegeneration, suggesting MyD88 is an endogenous pro-survival molecule in the brain. MyD88 deficiency led to enhanced ischemic injury in a manner independent of the pro-inflammatory pathway. Notably, MyD88 stabilized brain ischemia-induced desphoshorylation of CaMKII in the brain. The binding of MyD88 to protein phosphatase 1 (PP1) was significantly enhanced following oxygen glucose deprivation (OGD) treatment in vitro, suggesting that the MyD88 stabilizes CaMKII dephosphorylation via PP1. Taken together, we establish an interaction between MyD88 and CaMKII in brain ischemia tolerance, and provide a fresh insight into the basic and clinical research of cerebral ischemia.

Disclosures: **J. Ren:** None. **Y. He:** None. **K. Qian:** None. **Y. Shen:** None. **Y. Zhou:** None.

Poster

517. Ischemia: Molecular Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 517.26/FF2

Topic: C.07. Ischemia

Title: Cold-inducible RNA-binding motif protein 3 (RBM3) protects neural cells against hypoxic-ischemic (HI) injury and is involved in stemness maintenance of neural stem progenitor cells (NSPC)

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Abstract: Moderate hypothermia is a potent therapeutic tool to ameliorate hypoxic-ischemic (HI) brain injury. RNA-binding motif protein 3 (RBM3) belongs to a small group of proteins, whose expression is elevated during hypothermia while global protein translation is suppressed. Recently, RBM3 has been identified as a positive effector in hypothermia-mediated neuroprotection against HI injury, and is potentially involved in neurogenesis in euthermic condition. However, the underlying molecular mechanisms remain largely unknown.
We took advantage of an *ex vivo* organotypic hippocampal slice culture (OHSC) model from postnatal day 3 C57BL7/J mice, and challenged OHSC with oxygen-glucose deprivation (OGD)

to mimic HI injury. We observed that moderate hypothermia (32°C) significantly reduces OGD-induced neuronal cell death, and meanwhile increases RBM3 expression level, particularly in dentate gyrus (DG) where postnatal neurogenesis occurs.
Therefore, we have looked at expression levels of RBM3 in cultured NSPCs. We found high RBM3 expression in proliferating NSPCs which decreased dramatically when cells were stimulated to differentiate. Doublecortin, a marker for neuroblasts and immature neurons, was simultaneously upregulated. These results indicate that RBM3 may play an important role in the maintenance of NSPC stemness. Previously we performed an interactome screening and discovered protein interactions between RBM3 and oncofetal protein IMP family members, which are known to regulate NSPC properties and fetal neural development. Here we demonstrated that IMP1, IMP2 and IMP3 are abundant in NSPCs but diminish with differentiation, similar to the expression pattern of RBM3. Among the three IMP proteins, only IMP2 is expressed in postnatal brain. We confirmed the interaction of RBM3 and IMP2 *in vitro* and *in vivo*, by co-immunoprecipitation (CoIP) and proximity ligation assay (PLA), respectively. In summary, RBM3 may play a key role in hypothermia-mediated brain protection against HI injury, and is involved in regulating NSPC self-renewal by interacting with oncofetal protein IMP2.

Disclosures: X. Zhu: None. **C. Bregere:** None. **R. Guzman:** None. **J.P. Kapfhammer:** None. **S. Wellmann:** None.

Poster

517. Ischemia: Molecular Mechanisms

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Topic: C.07. Ischemia

Support: PDL National Institutes of Health GM55632

LL Research Foundation - Flanders (FWO)

MFA Heart & Stroke Foundation Canada (HSFC) fellowship

CCN holds a Canada Research Chair

Title: Targeting MAPK phosphorylation sites of the Connexin43 C-terminal tail provides neuroprotection in stroke

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Abstract: Connexin43 (Cx43) gap junction (GJ) channels directly bridge the cytoplasm between coupled astrocytes, and allow for direct intercellular signaling. However, Cx43 channels also exist on their own as single membrane hemichannels that connect the cell cytoplasm to the extracellular milieu. In brain injury, Cx43 GJ channels are largely associated with protective mechanisms while hemichannels are linked to deleterious effects. The cytoplasmic C-terminal region is a key factor in Cx43 function with putative phosphorylation sites specific to different kinases that directly mediate channel and hemichannel activity. The mitogen activated protein kinase (MAPK) has been linked with regulating Cx43 channel function. The goal of this study was to determine whether the MAPK phosphorylation sites of Cx43 are critical in stroke outcome.

We subjected wild-type (WT) and mutant (MK4) mice, with mutations within the Cx43 C-terminal that include the MAPK site Cx43(S255/262/279/282A), to permanent middle cerebral artery occlusion (pMCAO). After 4 days of recovery, brain sections were histologically evaluated for infarct volume and were correlated with behavioral outcomes. Immunofluorescent analysis of astrocyte reactivity, microglial activation and apoptosis were performed. Electrophysiological analysis of Cx43 hemichannel activity as well as Cx43 GJ coupling studies were performed in WT and MK4 astrocytes.

A significant decrease in infarct volume was measured in MK4 mice. In the penumbra, an increase in astrocyte reactivity as well as a decrease in microglial activation was observed in the MK4 mice, compared with WT animals. Consistent with the infarct volume data, a significant reduction in cell death was also observed in MK4 mice; this was correlated with significant improvement in behavioral performance. In contrast to WT astrocytes, MK4 astrocytes displayed a reduction in Cx43 hemichannel activity and an increase in GJ channel coupling. This unique channel characteristic is associated with the neuroprotective phenotype of MK4 animals to stroke. This was confirmed by injecting ischemic mice with TAT-GAP19, a nonapeptide derived from the cytoplasmic loop of Cx43 and previously shown to inhibit hemichannel activity without affecting GJ coupling (Abudara, V., et al. (2014), Front Cell Neurosci 8: 306; Wang et al., (2013) Basic Res Cardiol, 108: 309). Similarly to the MK4 phenotype, those mice treated with TAT-GAP19 showed a significant reduction in infarct volume compared to non-treated mice. Taken together, understanding the molecular mechanisms associated with Cx43 channel function in stroke, opens up new avenues for therapeutic intervention.

Disclosures: M. Freitas-Andrade: None. **N. Wang:** None. **J. Bechberger:** None. **P.D. Lampe:** None. **L. Leybaert:** None. **C.C. Naus:** None.

Poster

517. Ischemia: Molecular Mechanisms

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Program#/Poster#: 517.28/FF4

Topic: C.07. Ischemia

Support: AHA GRNT20410061

AHA SDG17140056

Title: Sesn3 and Post-Ischemia Seizures in Diabetes

Authors: *Z. C. XU, Z. LEI, L. XIA, Z. SHI;
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Abstract: Seizures are the most common neurological sequelaee of stroke. Diabetes mellitus has been identified as an independent predictor of acute seizures in stroke patients. The mechanisms of post-stroke seizures under diabetic condition remain unclear. Recent studies indicated that epilepsy could be mediated by energy metabolism related proteins such as Sestrin3 (Sesn3). The present study attempted to reveal the contribution of Sesn3 to seizure generation in diabetic condition after ischemia.
Transient global ischemia was produced in adult Wistar rats mice. Diabetes was induced by i.p. injection of 50 mg/kg streptozotocin (STZ). The seizure activity was defined by the Racine scale III-V. The neuronal death in the brain was determined by hematoxylin-eosin staining. The expression levels of Sesn3 were analyzed by Western blotting and immunohistochemistry. The neuronal excitability was recorded with electrophysiological approaches.
The blood glucose levels was >300 mg/dL in animals one week after STZ injection. The seizure rate significantly increased from zero of naive animals to 100% in diabetic rats 24 hr after 15 min ischemia. No obvious neuronal damage was observed in hippocampus and cerebral cortex one day after ischemia. Sesn3 expression in hippocampus was significantly increased in diabetic animals with post-ischemia seizures. The potassium currents were decreased and neuronal excitability increased in these animals. The seizure rate was decreased from 50% of wild type mice to 15% in Sesn3 knockout mice after 15 min ischemia. The above results indicate that increase of Sesn3 expression might be responsible to post-ischemia seizure in diabetic animals.

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Poster

517. Ischemia: Molecular Mechanisms

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Program#/Poster#: 517.29/FF5

Topic: C.07. Ischemia

Support: NIDCR Intramural Grant: DE000485-26

Title: Deficiency of Perlecan, a basement membrane heparan sulfate proteoglycan, leads to the blood-brain barrier deterioration in a mouse ischemic stroke model

Authors: *K. NAKAMURA[1,3], P. ZHANG[1], T. IKEUCHI[1], C. RHODES[1], D. MAHBOUBI[1], Y. CHIBA[1], T. AGO[3], Y.-S. MUKOUYAMA[2], Y. YAMADA[1];
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Abstract: Ischemic stroke is a leading cause of death and disability. The pathological process of ischemic stroke is worsened by the disruption of blood-brain barrier (BBB). The BBB is a highly selective permeability barrier formed by brain endothelial cells and covered by basement membranes (BMs), pericytes, and the end-feet of astrocytes. The expression of platelet-derived growth factor receptor β (PDGFRβ), a marker of pericyte, is increased around the ischemic lesion for the maintenance of the BBB and the involvement in the repair process. Here we focus on Perlecan, the major heparan sulfate proteoglycan in the BMs, which is preferentially expressed in endothelial cells in the brain. Previous studies demonstrate that Perlecan interacts with extracellular matrix, growth factors, and receptors, implicating various cellular activities and tissue homeostasis. However, the role of Perlecan in the BBB breakdown and the subsequent repair process after ischemic stroke remains unclear.
To elucidate the role of Perlecan in the brain vasculature, we used conditional *perlecan*-deficient (*Perlecan*$^{-/-}$-Tg) mice. The BBB formation and function in the brain vasculature appeared to be unaffected in *Perlecan*$^{-/-}$-Tg mice. However, under the ischemic stroke model produced by a transient middle cerebral artery occlusion, *Perlecan*$^{-/-}$-Tg mice exhibited larger infarct volumes and more BBB leakage than control mice on post-surgery day (PSD) 2. Moreover, *Perlecan*$^{-/-}$-Tg mice had less PDGFRβ-positive pericytes around the ischemic lesion on PSD 3 to 7, suggesting that the Perlecan deficiency results in defective pericyte activation during the repair process. Combined, these results suggest that Perlecan is required for the endothelial cell-pericyte integrity in the BBB maintenance and the pericyte activation in the BBB repair process after ischemic stroke.

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Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.01/FF6

Topic: C.08.Stroke

Support: National Research Foundation of Korea (NRF) Grant 2013R1A1A2074231, 2013R1A2A2A01067248

DGIST R&D Program of the Ministry of Science Grant 15-BD-0402

SSTF-BA1502-13

Title: Alternatively activated brain-infiltrating macrophages facilitate recovery from collagenase-induced intracerebral hemorrhage

Authors: *W. CHO[1], H. MIN[1], Y. JANG[1], I.-H. CHO[2], S.-W. YU[3], S. LEE[4], S. LEE[1]; [1]Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Kyung Hee Univ., Seoul, Korea, Republic of; [3]Daegu Gyeongbuk Inst. of Sci. and Technol., Daegu, Korea, Republic of; [4]Chungnam Natl. Univ., Daejeon, Korea, Republic of

Abstract: Intracerebral hemorrhage (ICH) is one of the major causes of stroke. After onset of ICH, massive infiltration of macrophages is detected in the peri-hematoma regions. Still, the function of these macrophages in ICH has not been completely elucidated. In a collagenase-induced ICH model, CX3CR1+ macrophages accumulated in the peri-hematoma region. Characterization of these macrophages revealed expression of alternatively activated (M2) macrophage markers. In the macrophage-depleted mice, ICH-induced brain lesion volume was larger and neurological deficits were more severe compared to those of control mice, indicating a protective role of these macrophages in ICH. In the ICH-injured brain, mannose receptor-expressing macrophages increased at a delayed time point after ICH, indicating M2 polarization of the brain-infiltrating macrophages in the brain microenvironment. To explore this possibility, bone marrow-derived macrophages (BMDM) were co-cultured with mouse brain glial cells and then tested for activation phenotype. Upon co-culture with glia, the number of mannose receptor-positive M2 macrophages was significantly increased. Furthermore, treatment with glia-conditioned media increased the number of BMDM of M2 phenotype. In this study, our data suggest that brain-infiltrating macrophages after ICH are polarized to the M2 phenotype by brain glial cells and thereby contribute to recovery from ICH injury.

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Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.02/FF7

Topic: C.08.Stroke

Support: NS092618 to X.H

Title: St2/il-33 signaling restricting acute ischemic brain injury involves switching microglia/macrophage polarization

Authors: *Q. YE, Y. YANG, H. ZHANG, J. WANG, G. WANG, J. CHEN, X. HU, X. HU; Neurol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: BACKGROUND AND PURPOSE: ST2/IL33 signaling is involved in immune regulation and inflammatory responses. It has been shown that both IL33 and ST2 are expressed in CNS and may play an important role in recovery after spinal cord injury. Recent study documented an increase in blood ST2 expression after stroke. The exact roles of ST2 in the ischemic brain injury, however, are not explored. **METHODS:** C57/BL6 wild type (WT) and ST2-KO mice were subjected to 60-minute transient cerebral ischemia (tMCAO) or distal permanent MCAO (dMCAO). The expression of ST2 and IL-33 in the ischemic brain were measured at different time points after tMCAO using RT-PCR. Brain infarct was quantified using TTC staining and MAP2 immunostaing. Neurobehavioral tests were performed up to 7 days after tMCAO. The expression of ST2 on CNS cells was assessed by flow cytometry analysis. For experiments with ST2 activation, IL-33 was injected intracerebroventricularly (i.c.v.) into WT mice right after the induction of tMCAO. **RESULTS:** In WT mice, the mRNA expression of ST2 in the ischemic brain exhibited a delayed elevation 7d after tMCAO, while the mRNA expression of IL-33 was transiently increased 1d after tMCAO. The deficiency of ST2 resulted in enlarged Infarct volume 3d after tMCAO or dMCAO and aggravated neurological deficits lasted out to 7d. Further study showed that i.c.v. injection of IL-33 at the time of reperfusion attenuated the severity of brain infarct in WT mice, suggesting that central activation of ST2 could protect against acute ischemic brain injury. Furthermore, immunofluorescent staining shows reduced microglia/macrophage polarization towards M2 phenotype in ST2 deficient mice. In vitro results confirm the direct effect of IL-33 of microglial polarization under physiological conditions or upon ischemic neuronal death. **CONCLUSIONS:** ST2/IL-33 signaling plays essential role in restricting acute ischemic brain injury. Such protective effect of ST2/IL-33 involves a shift of microglia/macrophage polarization towards M2 phenotype. Our study may provide a new therapeutic target for stroke treatment.

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Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.03/FF8

Topic: C.09. Brain Injury and Trauma

Support: R01-AG-033028

OSUCOM Dean's Discovery Grant

American Surgical Society Fellowship

NINDS P30-NS045758

Title: Rod microglia induced by traumatic brain injury are resident cells that align with blood vessels adjacent to damaged neurons

Authors: *K. G. WITCHER[1], B. N. BENNER[1], M. M. MUCCIGROSSO[1], D. B. MCKIM[1], A. M. FENN[1], J. LIFSHITZ[3], D. S. EIFERMAN[2], J. P. GODBOUT[1];
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Abstract: Traumatic brain injury (TBI) elicits immediate neuroinflammation that contributes to acute cognitive, motor, and affective disturbances. Although acute impairments resolve after mild to moderate TBI, inflammatory processes persist and may underlie neuropsychiatric and cognitive complications that arise long after injury. Microglia, the innate immune cells resident to the brain, are key mediators of acute and chronic inflammation. We have previously reported that methylene blue (MB), an antioxidant and anti-inflammatory agent, reduces microglia-mediated neuroinflammation after midline fluid percussion injury, a murine model of diffuse TBI. This was associated with improved recovery of motor coordination 7 days post-injury (dpi). In examining microglia morphology 7 dpi, we found that MB intervention prevented TBI-induced formation of rod microglia in the cortex. Although the formation of rod microglia after FPI has been observed, their origin and role in pathophysiology is unclear. Here we show novel data that rod microglia are in close proximity to axotomized (ATF-3+) neurons 7 dpi and form trains along nearby blood vessels. Furthermore, both rod microglia and nearby activated microglia upregulate CD45. We confirmed that CD45+ rod microglia originate from resident CNS cells (unlabeled in GFP bone marrow chimeras), but not through proliferation (unlabeled by BrdU). Overall, these findings suggest that TBI drives resident microglia to take on a rod morphology, align with blood vessels proximal to damaged cortical neurons, and that this process can be prevented by anti-inflammatory intervention. One possible interpretation is that

the rod morphology facilitates movement of microglia along blood vessels toward sites of neuronal injury.

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Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

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Topic: C.09. Brain Injury and Trauma

Support: NIH Grant 1RF1AG048083-01

DOD Grant W81XWH-15-1-0561

Title: Ex-vivo modeling of Alzheimer's Disease outcomes following traumatic mechanical injury of human iPSC-derived neurons

Authors: A. M. BALCER[1], R. DOS SANTOS CHAVES[2], E. GUTIERREZ[3], A. GROISMAN[3], L. S. B. GOLDSTEIN[4], A. ALMENAR-QUERALT[4], *S. B. SHAH[2];
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Abstract: Traumatic brain injury (TBI) is a risk factor for developing Alzheimer's Disease (AD), and occurs when the brain is mechanically loaded by an external force. Unfortunately, we do not yet understand why individuals develop AD following TBI. In light of the heterogeneous and complex neuronal architecture within the brain, and the focal and diffuse nature of traumatic lesions, we hypothesized that the geometry and orientation of axons and dendrites relative to the directionality of traumatic impact will dictate the severity of their structural changes and injury-induced AD phenotypes. This study details our initial deployment of a new *ex-vivo* cell injury model, which we used to probe morphological and biochemical phenotypes following rapid unidirectional deformation of human neurons derived from induced pluripotent stem cells (iPSC). Human iPSC-derived neuronal progenitor cells (NPCs) were either i) seeded and directly differentiated into neurons within stretching devices; ii) differentiated into neurons in standard culture plates, prior to seeding within devices after dissociation; or iii) differentiated into neurons in standard culture plates, prior to dissociation and FACS-sorting to enrich for a neuronal population. In all experimental groups, at time points up to 24 hours, we compared the response

of unstretched cells to those subjected to 1 or 50 cycles of 24% stretch followed by release. We found that neurites aligned along the axis of stretch experienced immediate and significantly more frequent and more severe structural changes in response to stretch, as defined by residual neurite waviness upon release (likely created by the inelastic lengthening of axons during stretch). How neurite length and thickness influence the severity of stretch-induced morphological changes was also determined. As a first step in addressing whether our mechanical injury model induces AD-related phenotypes, we used ELISA-based and immunocytochemistry assays to quantify accumulation of secreted amyloid beta peptide and changes in tau phosphorylation, two classical AD hallmarks, in response to rapid stretch. Our study demonstrates the feasibility of a powerful model system and represents the first steps towards developing a deeper mechanistic understanding of the linkage between TBI and AD.

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Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

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NEUREX

German Research Foundation Grants SCHA 1442/3-2 and SCHA 1442/5-1

Title: Fibrinogen regulates adult neural stem/precursor cell differentiation into astrocytes via BMP receptor signaling

Authors: ***C. SCHACHTRUP**[1], S. SCHILDGE[2], L. POUS[2], C. BOHRER[2], K. MAMMADZADA[2], D. PFEIFER[3], K. SCHACHTRUP[4], K. AKASSOGLOU[5,6];
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Germany; [5]Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [6]Dept. of Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Subventricular zone (SVZ)-derived neural stem/precursor cells (NSPCs) have the potential to contribute to brain repair upon CNS injury or disease. However, the majority of SVZ-derived NSPCs differentiate into glial cells or stay in a precursor state instead of differentiating into functionally integrated neurons. This hindrance of neuronal regeneration is mainly attributed to changes in the NSPC environment. Identification of the extracellular factors driving NSPC differentiation and characterizing the contribution of SVZ-derived newborn cells to brain repair is essential to harness endogenous NSPCs for therapeutic goals. Here, we show that the blood protein fibrinogen drives NSPC differentiation into astrocytes via activation of the BMP receptor signaling pathway. After cortical injury, we could show that fibrinogen is deposited not only in the cortical lesion area, but also rapidly in the NSPC microenvironment within the SVZ niche. Interestingly, in fibrinogen knockout mice, the total number of SVZ-derived newborn astrocytes was reduced by 50 % in the body of the cortical lesion center. Accordingly, stereotactic injection of fibrinogen into the mouse cortex resulted in a 30 % increase in the total number of SVZ-derived astrocytes, revealing a specific role for fibrinogen driving adult NSPC differentiation into astrocytes. Fibrinogen-mediated differentiation of NSPCs into astrocytes is reversed by blocking β1 integrin-mediated BMP receptor recruitment into lipid rafts or by blocking phosphorylation of the BMP receptor. Microarray and protein expression analyses of fibrinogen-induced NSPCs differentiating into newborn astrocytes revealed a distinct astrocyte signature compared to resident cortical astrocytes, with a potential beneficial function after brain repair. These results identify fibrinogen as a novel environmental factor of adult NSPCs that induces BMP receptor signaling leading to NSPC differentiation into astrocytes after traumatic brain injury.

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Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

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Program#/Poster#: 518.06/FF11

Topic: C.09. Brain Injury and Trauma

Title: Molecular, cellular, and behavioral improvements after treatment of rodent traumatic brain injury with human mesenchymal stem cells.

Authors: *C. W. LEVENSON, A. DARKAZALLI, C. VIED;
Florida State Univ. Col. of Med., Tallahassee, FL

Abstract: Mesenchymal stem cells have emerged as a promising therapy to reduce secondary injury and cognitive deficits associated with traumatic brain injury (TBI). To identify mechanisms responsible for the efficacy of stem cell-based therapies, we employed a rodent model of TBI (controlled cortical impact), combined with behavioral testing, high throughput RNA sequencing (RNAseq), and an examination of the role of endogenous neural progenitor cells after treatment with $1x10^6$ intravenously delivered adult human bone marrow-derived mesenchymal stem cells (hMSC). RNAseq and KEGG pathway analysis revealed that TBI altered the abundance of almost 7000 mRNAs regulating pathways involved in metabolism, neuronal plasticity, receptor-mediated cell signaling, and neurodegenerative diseases including Parkinson's and Alzheimer's. hMSC treatment normalized 50% of these TBI-disrupted genes, with some pathways exhibiting 100% normalization by this cell-based therapy. We also investigated a possible role for hMSC in the role and regulation of endogenous neural progenitor cells that arise from in the lateral end of the subventricular zone (SVZ). Stereological analysis revealed that within 48h, TBI produced a 52% ($p \leq 0.05$) increase in the number of proliferating cells in the SVZ. Treatment with hMSC resulted in a further 74% ($p < 0.001$) increase in proliferation. Consistent with human studies, TBI resulted in depression-like behaviors as measured by a 54% ($p \leq 0.05$) decrease in saccharin preference scores. Treatment with hMSC fully prevented TBI-associated depression. TBI was also found to produce a 73% ($p \leq 0.05$) decrease in novel object interaction time, indicating impaired working memory that was similarly prevented by hMSC treatment. To test the hypothesis that hMSC efficacy is dependent cell proliferation in the SVZ, precision X-ray irradiation was used to selectively eliminate mitosis in the SVZ prior to hMSC treatment. The ability of hMSC to prevent TBI-associated depression and working memory impairment was eliminated when proliferation in the SVZ was inhibited by targeted irradiation, suggesting a key role for these cells in the efficacy of hMSC in this model. Together this work not only shows that hMSC may be an effective treatment option for TBI-induced depression and cognitive deficits, but also identifies a number of novel molecular targets for the development of new therapeutic approaches.

Disclosures: C.W. Levenson: None. **A. Darkazalli:** None. **C. Vied:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.07/FF12

Topic: C.09. Brain Injury and Trauma

Support: NINDS NS083064

Title: Characterization and modulation of stem-like cells after a controlled cortical impact injury in mice

Authors: *B. R. DESOUSA[1], A. AHMED[1,2], M. SPURLOCK[1], S. GAJAVELLI[1], R. BULLOCK[1];
[1]Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL; [2]Dept. of Neurosurg., Univ. Hosp. Southampton, Southampton, United Kingdom

Abstract: Traumatic Brain Injury (TBI) often leads to disability among survivors as the brain fails to regenerate. Ethical and immune compatibility issues limit the use of exogenous cell transplant approaches. Promoting regeneration of injured nervous system via endogenous neural stem cells (NSCs) is becoming possible. To harness these cells for repair they have to exit quiescence and be driven toward neuronal and glial lineages. In uninjured cortex such cells are absent, as evidenced by proliferative potential under in vitro conditions. In contrast, cortical glial fibrillary acidic protein (GFAP) cells/reactive astrocytes acquire stem properties following injury. Studies have linked the stem cell response of the astrocytes to the Sonic Hedgehog (SHH) signaling pathway. We hypothesized that (1) controlled cortical impact (CCI) injury will modify the activated stem-like cells in the cortex and (2) that modulation of the SHH pathway will alter functional outcome. In this study, we show that stem-like cells in the cortical parenchyma are activated following CCI injury, and the quantity of such cells can be modulated through the SHH signaling pathway. Activation of cortical cells was determined using a Neurosphere stemness assay and immunohistochemistry to assess the proliferative and differentiating potential for these cells. We showed that a significant number of neurospheres, or stem-like cells, were produced at 3 and 7 days post-injury (DPI). These stem-like cells were also shown to have the capacity to become neurons, oligodendrocytes, and astrocytes. We also show that upregulation of the SHH signaling pathway improves function after cortical injury. Animals were treated with a modulator of the SHH signaling pathway, either Smoothened agonist (SAG) or Cyclopamine (SHH antagonist), and motor function was assessed using the Rotarod performance test. Mixed ANOVA analysis suggests a significant effect at 3, 7, 14, and 28 DPI when treated with SAG, but not Cyclopamine, as the animals were able to remain on the rotating rod for a longer period of time when treated with SAG. From this study, we determined that (1) stem-like cells are activated after CCI injury in the cortex with the most being activated at 3 DPI, and that (2) modulation of the SHH signaling pathway with SAG improved motor function at 3, 7, 14, and 28 DPI.

Disclosures: B.R. Desousa: None. **A. Ahmed:** None. **M. Spurlock:** None. **S. Gajavelli:** None. **R. Bullock:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.08/FF13

Topic: C.09. Brain Injury and Trauma

Title: Neural stem cell transplants promote cortical reorganization after traumatic brain injury

Authors: *C. P. ADDINGTON, S. E. STABENFELDT, J. A. KLEIM;
Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ

Abstract: Traumatic brain injury (TBI) afflicts over 1.7 million Americans and leads to 52,000 deaths annually. TBI is characterized by a primary, mechanical insult that leads to an expansive secondary, biochemical injury that is largely responsible for long-term deficit associated with TBI. Despite the prevalence of TBI and associated impairments, there are currently no clinical therapies that directly address the secondary injury of TBI. Preclinical studies have used stem cells to mitigate the deleterious effects of TBI with moderate success; however, the mechanism of benefit of cell therapies after TBI remains unclear. We have used intracortical microstimulation to investigate the capacity of neural progenitor/stem cell (NPSC) transplants to modulate cortical motor map plasticity in a rat model of TBI. We observed NPSC transplants to promote significant preservation of forelimb motor map areas at subacute time points after TBI compared to vehicle-receiving controls ($p=0.0227$ at 7 days after TBI). However, the ratio of distal to proximal forelimb representation is widely varied in NSPC-mediated motor map recovery. These data indicate that stem cell therapies may represent a mechanism for promoting functional reorganization within residual cortical tissue after TBI.

Disclosures: C.P. Addington: None. **S.E. Stabenfeldt:** None. **J.A. Kleim:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.09/FF14

Topic: C.08.Stroke

Support: NIH R01 NS073815

Title: Microglial proliferation after stroke is indirectly controlled by ETB_R signaling in reactive astrocytes.

Authors: J. J. MCINNIS, M. D. LECOMTE, *J. L. SPEES;
Med., Univ. of Vermont, Colchester, VT

Abstract: During central nervous system injuries such as stroke, the glial response includes both reactive astrocytes ($GFAP^+$) and activated microglia ($CD11b^+$). Within the injury environment, paracrine effectors released by reactive astrocytes and activated microglia are thought to cross-activate both glial cell types. In culture, many factors secreted by glial cells are reported to promote glial activation in an autocrine/paracrine manner. However, few studies have clearly demonstrated such reactive astrocyte/microglial interactions *in vivo*. Previous work from our lab demonstrated a Notch1-STAT3- ETB_R signaling axis that controls reactive astrocyte proliferation after brain injury. In addition to proliferation, this signaling axis may also control reactive astrocyte function(s). Here we show that disruption of ETB_R signaling in astrocytes using inducible, conditional knockout (cKO) mice (GFAP-$CreER^{TM}$-ETB_R-cKO), leads to a significant reduction in microglial proliferation in the peri-infarct area after stroke as measured by $CD11b^+/Ki67^+$ cells. Through proteomic studies on ETB_R-cKO astrocytes, we are currently performing screens to identify factors released by proliferating reactive astrocytes that regulate microglial activation and proliferation.

Disclosures: J.J. McInnis: None. **M.D. LeComte:** None. **J.L. Spees:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.10/FF15

Topic: C.08.Stroke

Title: Modulation of microglial polarization and phagocytosis by hemoglobin in an *Ex vivo* model of intracerebral hemorrhage

Authors: *Q. LI, X. LAN, X. HAN, J. WANG;
Anesthesiol. & Critical Care Med., Johns Hopkins Univ., Baltimore, MD

Abstract: Background and Purpose: Microglia are activated in the peri-hematoma region after intracerebral hemorrhage (ICH). The classically activated microglia (M1) release inflammatory factors, whereas alternatively activated microglia (M2) carry out anti-inflammatory functions and phagocytize hematoma and debris. We aimed to investigate whether hemoglobin regulates

polarization of and phagocytosis by microglia in an *ex vivo* model of ICH. **Methods:** Hemoglobin (Hb), a main metabolite of blood, was used to mimic ICH *ex vivo*. We treated organotypic hippocampal slice cultures (OHSCs) with Hb (20 μM) or vehicle for 16 h and collected tissue to examine the protein/mRNA levels of M1 (CD11b, iNOS, and CD16/32) and M2 (Arg1, IL-10) markers and examine cytokines and chemokines in the conditioned media. We also treated OHSCs with Hb or vehicle for 16 h and performed a phagocytosis assay using fluorescent-conjugated latex beads or aged red blood cells from GFP-UBC mice. **Results:** In cultured mouse OHSCs, Hb induced *CD11b, iNOS,* and *Arg1* ($p<0.05$) mRNA expression; induced CD11b, CD16/32, and Arg1 protein expression; and enhanced the expression/secretion of IL-10 ($p<0.05$) and other M1 markers (IL-6 and TNF-α, $p<0.05$). In a cytokine/chemokine array performed using Hb- or vehicle-treated media from OHSCs, multiple inflammation related cytokines/chemokines were increased by Hb, as well as the anti-inflammation factor IL-10. In addition, we found that treating OHSCs with Hb increased microglial phagocytosis ($p<0.05$). **Conclusion:** Hb treatment *ex vivo* induced overexpression of pro- and anti-inflammatory factors and increased phagocytosis of microglia. OHSCs can be used as an *ex vivo* platform to study microglial polarization and phagocytosis after ICH.

Disclosures: Q. Li: None. **X. Lan:** None. **X. Han:** None. **J. Wang:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.11/FF16

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant HD061963

Title: Anxiety-related and depression-like behaviors following mild traumatic brain injury in adolescent rats are dependent on sex and estrous cycle

Authors: *L. L. KRAFJACK[1], R. RAGHUPATHI[2];
[2]Neurobio. & Anat., [1]Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Following mild traumatic brain injury (TBI), high school and collegiate-aged female athletes tend to report more emotional symptoms than male athletes. Thirty five-day-old male and female rats were used to model mild TBI in the adolescent human. Rats were anesthetized and directly impacted on the skull using a 5mm metal tip just behind bregma over the midline suture (2mm depth, 5.5m/s velocity); sham-injured rats were anesthetized, but not injured. Injury resulted in similar percentages (90% of males, 85% of females) of skull fractures and righting

reflex times in male (473 ± 28 sec) and female (463 ± 20 sec) rats, which was significantly different ($p<0.05$) than their sham-injured, age-matched counterparts (male, 269 ± 28 sec; female, 270 ± 28 sec). Brain-injured male and female rats were evaluated for anxiety-related and depression-like behaviors using the elevated plus maze (EPM) and forced swim test (FST), respectively, at 6 weeks. Male rats did not exhibit any changes in open arm time in the EPM or immobility time in the FST at 6 weeks post-injury. Vaginal cytology was performed to determine the phase of the estrous cycle females rats were in at the time of behavioral testing. During the estrus phase, when circulating levels of estradiol and progesterone are low, brain-injured female rats spent more time in the open arm of the EPM compared to their phase-matched, sham-injured counterparts. During the proestrus phase, when circulating estradiol is high, brain- injured animals exhibited decreased open arm time, suggestive of an anxiety-related phenotype. Open arm time in the EPM during the diestrus phase, when circulating levels of progesterone are high, did not differ between sham- and brain-injured rats. Brain-injured rats also exhibited increased immobility in the forced swim test during the estrus and diestrus phases, suggestive of a depression-like phenotype, but immobility time did not differ between sham and brain-injured rats during the proestrus phase. Injured brains were evaluated for neuronal damage (NeuN), neurodegeneration (FluoroJade), astrocytic (GFAP) and microglial (Iba1) reactivity at 6 weeks post-injury. Immunoreactivities for NeuN, GFAP, or Iba1 were not different between sham- and brain-injured animals at 6 weeks post-injury suggestive of the mild nature of the trauma. In contrast, both male and female brain-injured rats exhibited a similar degree of axonal degeneration in the corpus callosum. Taken together, these data suggest that female rats may be more vulnerable to anxiety-related and depression-like symptoms following mild TBI and that future behavioral analyses using female rats in TBI should take the estrous cycle into account.

Disclosures: L.L. Krafjack: None. **R. Raghupathi:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.12/FF17

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant HD061963

Title: Changes in cortical activity may underlie motor deficits and seizure susceptibility following traumatic brain injury in the neonate rat

Authors: *L. A. HANLON[1], J. W. HUH[2], R. RAGHUPATHI[1];
[1]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Traumatic brain injury (TBI) in infants results in long-lasting deficits in cognition and motor function that are associated with structural alterations in the brain such as atrophy and ex vacuo ventriculomegaly. Both the cognitive deficits and the cellular pathologies have been replicated in a rat model of infant TBI. Furthermore, closed head impact over the left parietal cortex of 11-day-old rats produced sustained neurodegeneration and microglial reactivity in the injured cortex that preceded the cortical atrophy and enlargement of the lateral ventricles at 4 weeks post-injury. To investigate whether these pathologies affected neuronal activity, evoked field potentials (EFPs) were recorded at 3, 7, and 21 days post-injury by stimulating cells in layers 2/3 and recording from cells in layer 5 of the motor cortex in slices from the injured hemisphere. At 3 days post-injury, the amplitudes of the EFPs were significantly smaller in brain-injured animals (45%, $p<0.05$ compared to sham-injured controls). This hypoactivity was transient because at 7 and 21 days post-injury, the amplitudes of the EFPs of brain-injured animals were indistinguishable from those in the sham-injured, age-matched counterparts. Interestingly, post-injury administration of the antibiotic minocycline (45mg/kg/injection, 2x/day for 3 days) rescued the cortical hypoactivity at 3 days post-injury, but did not have any effect on injury-induced spatial learning and memory deficits on days 11-15 post-injury. Whereas the swim speeds of brain- and sham-injured animals were not different, a closer investigation using a straight-path swim task at 28 days post-injury revealed that brain-injured animals used the forelimb contralateral to the impact side to a significantly greater extent compared to sham-injured rats ($p<0.05$) . This deficit in motor control was preceded by a 104% increase in the amplitude of the EFP compared to sham-injured controls within the forelimb motor cortex - a region remote from the impact site wherein neurodegeneration or microglial reactivity were not observed. Additionally, brain-injured animals demonstrated at 14% decrease in their latencies to seize compared to sham-injured rats indicative of an increase in seizure susceptibility at 6 weeks post-injury. Both behavioral and neuronal activity deficits were independent of the sex of the brain-injured animal. Collectively, these data provide evidence of the complex underpinnings of the functional deficits observed in the immature brain in the acute and chronic post-traumatic periods.

Disclosures: L.A. Hanlon: None. **J.W. Huh:** None. **R. Raghupathi:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.13/FF18

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant HD061963

Title: Mild traumatic brain injury in adolescent rats results in sex-specific cognitive deficits

Authors: *R. RAGHUPATHI, L. L. KRAFJACK;
Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Following mild traumatic brain injury (TBI), girls perform more poorly on visual memory tasks and take longer to recover from their symptoms than their male counterparts. Thirty five-day-old male and female rats were used to model mild TBI in the adolescent human. Rats were anesthetized and impacted on the exposed skull using a 5mm metal tip just behind bregma over the midline suture (2mm depth, 5.5m/s velocity); sham-injured rats were anesthetized, but not injured. Injury resulted in similar percentages (90% of males, 85% of females) of skull fractures; righting reflex times in male (485 ± 18 sec) and female (471 ± 18 sec) brain-injured rats was significantly different than their sham-injured counterparts (male, 307 ± 23 sec; female, 286 ± 28 sec, $p<0.05$). Neither male nor female brain-injured rats exhibited deficits in spatial working memory in a Morris water maze-based task on days 4-7 post-injury. Brain-injured male rats did not exhibit deficits in novel object recognition (NOR) at 3, 8, or 28 days using either a 1hr or 24hr interval between the habituation and the testing trials. In contrast, brain-injured female rats exhibited a short term memory deficit in NOR at 3 days post-injury using the 1hr interval ($p<0.01$), but this deficit was no longer present at 8 days. Brain-injured female rats also exhibited a long term memory deficit in NOR using a 24hr interval at both 3 and 8 days ($p<0.01$), but this deficit was no longer present at 28 days. Injured brains were evaluated for neuronal damage (NeuN), neurodegeneration (FluoroJade), astrocytic (GFAP) and microglial (Iba1) reactivity, and blood brain barrier damage (IgG extravasation) at 3 and 8 days post-injury. NeuN immunoreactivity was unchanged in the brain-injured rats compared to their age-matched, sham-injured counterparts; no evidence of IgG extravasation at either 3 or 8 days was present suggesting that the blood-brain barrier was intact. Both male and female brain-injured rats exhibited similar degrees of axonal degeneration in the retrosplenial cortex and corpus callosum. Increased microglial reactivity was observed in the retrosplenial cortex and corpus callosum of both male and female brain-injured rats at 3 days post-injury ($p<0.01$), but returned to sham-injured levels by 8 days. Increased astrocytic reactivity was observed at 8 days post-injury in the retrosplenial cortex ($p<0.01$) and corpus callosum ($p<0.01$) in female rats only. Collectively, these data suggest that specific cognitive deficits following mTBI may be more prevalent in female rats, and that cellular pathologies may not be indicative of behavioral alterations.

Disclosures: **R. Raghupathi:** None. **L.L. Krafjack:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.14/GG1

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant HD061963

Title: Depletion of microglia immediately following traumatic brain injury in the neonate rat: Implications for cellular and behavioral pathology.

Authors: *J. W. HUH[1], L. HANLON[2], R. RAGHUPATHI[2];
[1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: In the immature rat brain, traumatic brain injury (TBI) results in cell death, impaired axonal transport (IAT) and axonal degeneration which are accompanied by activation of microglia. Eleven-day-old rat pups received an impact to the intact skull centered over the left parietal cortex; 24 hours later, both sham-injured and brain-injured animals received intra-cortical and intra-thalamic injections of either liposome-encapsulated sodium clodronate (which depletes microglia) or empty liposomes and brains were histologically evaluated at 3, 15, and 35 days post-injury. Clodronate depleted microglia in the cortex, white matter, and thalamus, and also decreased astrocyte density. In sham-injured animals, microglia and astrocyte densities were returned to sham-injured levels by 14 days post-injury. In brain-injured animals that received clodronate, were decreased up to 35 days post-injury compared to brain-injured animals that received the empty liposomes, suggestive of a deficit in repopulation. This sustained decrease in glia was accompanied by an increase in Fluoro Jade B-positive (FJB+) profiles in both the cortex and thalamus of clodronate-treated brain-injured animals at 3 ($p<0.001$), 14 ($p<0.01$), and 35 days post-injury, suggestive of a lack of clearance of degenerating cells. Clodronate administration had no effect on injury-induced IAT or axonal degeneration at 3 days post-injury suggesting that glia may not be involved in axonal injury. Clodronate-treated sham- and brain-injured animals and empty liposome-treated brain-injured animals were unable to perform the spatial learning task in the acute post injury period (days 10-14) as efficiently as sham-injured animals treated with the empty liposomes ($p=0.08$), implicating a role for glial cells in the development of the circuitry necessary for learning. In the chronic post-injury period (days 28-32), all brain-injured animals had spatial learning deficits ($p<0.05$) and clodronate-treated sham-injured animals were indistinguishable from sham-injured animals that received the empty liposomes. Collectively, these data suggest that microglia may predominantly play a phagocytic role in post-injury pathology. It remains to be seen, however, whether this lack of clearance has any effect on functional outcomes in the chronic post-injury period.

Disclosures: **J.W. Huh:** None. **L. Hanlon:** None. **R. Raghupathi:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.15/GG2

Topic: C.08.Stroke

Support: Heart and Stroke Foundation of Canada

Canadian Institutes of Health Research (CIHR)

Title: Microglia and infiltrating peripheral macrophages express different inflammatory markers after experimental permanent brain ischemia

Authors: ***J. G. ZARRUK**, A. D. GREENHALGH, S. DAVID;
Ctr. for Res. in Neurosci., McGill Univ. Hlth. Ctr., Montreal, QC, Canada

Abstract: Microglia and macrophages from the peripheral circulation contribute importantly to the inflammatory response after ischemic stroke. Recent studies have examined macrophage/microglia polarization in brain ischemia, however these studies have not distinguished between microglia and infiltrating macrophages. We used the LysM-EGFP knockin mouse in which myeloid cells (monocyte-derived macrophages and neutrophils) are tagged with EGFP which distinguishes them from microglia at sites of CNS damage. This was combined with immunostaining for P2ry12, a microglia specific marker. We studied the expression of pro-inflammatory and anti-inflammatory markers after a permanent Middle Cerebral Artery Occlusion (pMCAO). Immunostaining 3 days post-pMCAO brains with P2ry12, Iba-1, EGFP confirmed that the EGFP+/Iba-1+ cells were macrophages, while P2ry12+/Iba-1+ microglia did not express EGFP. The peak of EGFP+ myeloid cell infiltration was 72h post-ischemia and these cells were distributed evenly within the lesion core. FACS analysis showed that a significantly higher percentage of microglia expressed TNF-α at 3 and 7 days post-pMCAO compared with infiltrating macrophages. We then isolated microglia from infiltrating macrophages using FACS-sorting 72h post-ischemia to quantify the mRNA expression of pro and anti-inflammatory markers. We confirmed the purity of the extraction by quantifying *fcrls* (microglia specific gene) expression. Compared to microglia, infiltrating macrophages upregulated the expression of arginase-1 and Ym1 by 1000-fold, and the expression of CD206 by 10-fold. There was also a 100-fold increase in the expression of IL1-β in macrophages and no differences in TNF-α mRNA expression at this time point. Although MIP-1α was downregulated in ipsilateral microglia compared to contralateral microglia, a higher percentage of microglia

expressed this pro-inflammatory marker compared with macrophages. Finally, we confirmed by immunofluorescence that arginase-1 was only expressed in macrophages 72h post-pMCAO. These data show differences in the inflammatory expression profile between microglia and infiltrating macrophages after permanent brain ischemia. These results also show that microglia have a more proinflammatory profile as compared to macrophages suggesting that these two cell types play different and complementary roles after cerebral ischemic lesion.

Disclosures: J.G. Zarruk: None. **A.D. Greenhalgh:** None. **S. David:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.16/GG3

Topic: C.09. Brain Injury and Trauma

Support: KSCHIRT Grant 14-12A

NIH RO1 NS072302-02S1

NIH RO1 NS0072302

NIH T32 NS077889

NIH P30 NS051220

Title: Insulin-like growth factor1 overexpression mediates long-term survival and migration of immature neurons in the hippocampus following TBI

Authors: *E. LITTLEJOHN[1], S. K. MADATHIL[2], T. STEWART[1], K. SAATMAN[1]; [1]SCoBIRC, Univ. of Kentucky, Lexington, KY; [2]Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: The pathology associated with traumatic brain injury (TBI) manifests in motor and cognitive dysfunction following injury. Immature neurons residing in the neurogenic niche of the dentate gyrus (DG) in the hippocampus are particularly vulnerable to TBI. The inability to restore this population of hippocampal immature neurons following TBI has been causally linked to cognitive impairment. We have shown that elevating brain levels of IGF-1 stimulates hippocampal neurogenesis, enhancing the recovery of immature neuron numbers after severe TBI in mice. However, little is known about the effectiveness of IGF-1 to promote long-term survival or appropriate development of neurons born after injury. To this end, astrocyte-specific

IGF-1 conditionally overexpressing mice (IGF-1 TG) and wild-type (WT) mice received controlled cortical impact (n=9/genotype) or sham (n= 2/genotype) injury and 50 mg/kg BrdU (i.p.) twice daily for 7 days following TBI. At six weeks following injury IGF-1 significantly increased $NeuN^+/BrdU^+$ cell density in the granule cell layer. IGF-1 appropriately restricted the localization of these cells primarily to the inner 1/3 of the granule cell layer and restored the proportion of post-trauma proliferated cells that are $NeuN^+$ to a level equivalent to sham. The signaling mechanism through which IGF-1 modulates brain plasticity in the setting of TBI remains unclear. In the nervous system, PI3-K/Akt signaling predominates in mediating many of IGF-1 functions. Akt activation results in the phosphorylation of multiple downstream signaling molecules including mammalian target of rapamycin (mTOR). We hypothesized that increased brain levels of IGF-1 would potentiate posttraumatic activation of the mTOR signaling pathway, a pathway associated with growth and differentiation. To this end, IGF-1 TG and WT mice received CCI (n=8/genotype) or sham (n= 3/genotype) injuries. At 72hrs following injury, immunohistochemical labeling of pS6, a downstream effector of mTOR, was quantified in the granule cell layer, molecular layer, and the hilus of the dentate gyrus. Analysis of pS6 at the injury epicenter suggests that IGF-1 stimulates activity of the mTOR pathway following TBI. These data show that IGF-1 affects long-term survival and localization of posttrauma-born neurons. Further work is required to determine if this is appropriate or maladaptive and if mTOR is required for these effects.

Disclosures: E. Littlejohn: None. **S.K. Madathil:** None. **T. Stewart:** None. **K. Saatman:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.17/GG4

Topic: C.09. Brain Injury and Trauma

Title: Co-ultra pealut enhances neuronal recovery after a moderate traumatic brain injury

Authors: *S. CUZZOCREA, I. PATERNITI, M. CAMPOLO, R. CRUPI, M. CORDARO, G. BRUSCHETTA, R. SIRACUSA, E. ESPOSITO;
Univ. of Messina, Messina, Italy

Abstract: Traumatic brain injury (TBI) is a major health problem worldwide. Currently, there is no effective treatment to improve neural structural repair and functional recovery. Recently, has been demonstrated that hippocampal injury-induced neurogenesis is one mechanism underlying endogenous repair following TBI. The aim of this study was to evaluate the neuro-regenerative

properties co-ultra PEALut (constituted by the association of palmitoylethanolamide (PEA), with the flavonoid luteolin (Lut)) in a mouse model of TBI until 7 days. TBI was induced in mice by the controlled cortical impact (CCI), one of the most common models of TBI; the mice were orally administered co-ultra PEALut (1mg/Kg) 1h after trauma and daily for 72h and 7d. A conspicuous neurogenesis was seen in mice 72h and 7d after trauma. Co-ultra PEALut treatment stimulated neuronal reconstitution process restoring the basal level of new neurons (by BrdU staining) and mature neurons (by doublecortin staining). Moreover this positive effect was associated with an important upregulation of neurotrophic factors (BDNF and NT-3), ultimately leading to improvement in memory recall on behavioral testing (Morris water maze test). Co-ultra PEALut could represent a therapeutic potential of augmenting the endogenous repair response for treating TBI.

Disclosures: S. Cuzzocrea: None. **I. Paterniti:** None. **M. Campolo:** None. **R. Crupi:** None. **M. Cordaro:** None. **G. Bruschetta:** None. **R. Siracusa:** None. **E. Esposito:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.18/GG5

Topic: C.09. Brain Injury and Trauma

Support: The Moody Project for Translational Traumatic Brain Injury Research

Title: Effect of Nano Pulsed Laser Therapy (NPLT) on neurogenesis in a rat model of traumatic brain injury

Authors: *E. MOCCIARO[1,3], R. ESENALIEV[2], I. PETROV[2], Y. Y. PETROV[2], M. O. PARSLEY[1], D. S. DEWITT[1], D. S. PROUGH[1], M. A. MICCI[1];
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Abstract: Background: Traumatic brain injury (TBI) is a chronic disease that occurs after a head trauma and results in neurological dysfunctions. Neurobiology, in the last few years, has focused on the study of “neurogenesis” subverting the old thinking of the inability of the brain to replace neurons. It is now well accepted that in the adult brain, new neurons are continuously generated in the subventricular zone of the lateral ventricles and in the dentate gyrus of the hippocampus (an area of the brain involved in learning and memory and mostly affected by TBI). In previous studies we have shown that transcranial delivery of Nano-Pulsed Laser Therapy (SPLT), that combines the benefits of near-infrared laser light (808 nm) and of

ultrasound waves (generated with each short high-energy laser pulse within the tissue), is neuroprotective in a rat model of TBI (blast-induced neurotrauma; BINT). The aim of this work was to study the ability of NPLT to stimulate the proliferation and neuronal differentiation of hippocampal NSC in a rat model of TBI. **Methods:** For the *in vivo* studies, anesthetized adult male Sprague-Dawley rats were subjected to BINT or Sham injury. NPLT was applied 1 hour after BINT for 5 minutes. Proliferation of NSC in the hippocampus was studied using BrdU incorporation. In *in vitro* studies, hippocampal-derived NSC (Hipp-NSC), isolated from adult rat brains, were cultured and treated with nano pulse near-infrared laser light (NIL) alone or ultrasound (US) alone for 5 minutes. Proliferation was assayed using the MTS assay 1 and 2 days post-treatment. For differentiation studies, after US treatment, Hipp-NSC were plated onto poly-ornithine/laminin-coated plates and cultured for 7 days. The expression of neuronal (βIII-tubulin) and glial (GFAP) markers was assayed by immunofluorescence analysis. **Results:** NPLT increases the number of proliferating (BrdU+) Hipp-NSC in the hippocampus 7 days after BINT as compared to Sham rats. *In vitro*, US stimulation increased Hipp-NSC proliferation in a dose-dependent manner, and decreased the expression of neuronal markers but not of glial markers. NIL stimulation alone did not affect Hipp-NSC proliferation. **Conclusion:** Our results show that NPLT increases proliferation of NSC after BINT via generation of ultrasound waves, while NIL is likely to increase their neuronal differentiation.

Disclosures: E. Mocciaro: None. **R. Esenaliev:** None. **I. Petrov:** None. **Y.Y. Petrov:** None. **M.O. Parsley:** None. **D.S. DeWitt:** None. **D.S. Prough:** None. **M.A. Micci:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.19/GG6

Topic: C.08.Stroke

Title: Combined stem cell therapy for white matter stroke

Authors: *D. R. MILLER[1], J. A. MAZZITELLI[1], I. L. LLORENTE[1], W. E. LOWRY[2], J. CINKORNPUMIN[2], S. T. CARMICHAEL[1];
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Abstract: Stroke is currently the most prevalent neurological disease. Up to 800,000 people a year experience a first-time stroke and few completely recover. Subcortical white matter stroke (WMS) constitutes up to 30% of all stroke subtypes and is the second leading cause of dementia. Using a subcortical WMS mouse model with a large infarct area that mimics the larger white matter lesions seen in moderate to advanced human white matter ischemia on immunodeficient

NSG mice, the model is amenable to human cell transplantation. Two different cell lines were simultaneously injected into the infarct area: human induced pluripotent stem cells (iPSCs) - Neuronal progentitor cells (NPCs) as well as human iPSCs - glial enriched progenitor cells (GEPsa). iPSC GEPs produce immature glial cells and are therefore suited for WMS treatment. iPS cells were virally transduced with fluorescent reporters. Cells were injected 7 days after the stroke during the subacute stage in one injection of 100 000 cell/µl using a 1:1 ratio of iPS-NPCs and iPS-GEPs (50 000 each cell/µl). Immunohistochemistical staining was used to track the location, proliferation, and migration of the cells. Additionally, motor behavior was assessed using two different tasks. The gridwalking test assesses gait by placing animals on a mesh floor and measuring precision of foot placement. The cylinder test assesses spontaneous forelimb use and motor control during exploratory behavior. Axonal tracing of the fluorescent reporter expression indicated an increase of axons in the infarct area coming from both endogenous neurons and from the transplanted NPCs. Behavioral data showed an increase in motor function for the stem/progenitor cell transplantation group relative to the stroke group. These studies suggest that the transplantation of stem/progenitor cells in a combined therapy in moderate to advanced white matter stroke, as seen in vascular dementia, may provide a therapy to promote white matter repair and recovery.

Disclosures: D.R. Miller: None. **J.A. Mazzitelli:** None. **I.L. Llorente:** None. **W.E. Lowry:** None. **J. Cinkornpumin:** None. **S.T. Carmichael:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.20/GG7

Topic: C.08.Stroke

Support: NRF-2015M3A9B4067067

Title: Modified MSCs enhance functional recovery and neuroplasticity in chronic stroke via mechanisms involving angiogenesis, neurogenesis and anti-fibrosis.

Authors: *S. MARASINI, S.-W. YOO, D. CHANG, Y.-D. LEE, S.-S. KIM, H. SUH-KIM; Sch. of Medicine, Ajou Univ., Suwon, Korea, Republic of

Abstract: Stroke is a dominant cause of sensorimotor disability. Due to a limited or partial spontaneous functional recovery, stroke survivors often suffer from sensory-motor dysfunctions and various degrees of paralysis for several subsequent years. We previously reported that in acute phase of stroke, mesenchymal stem cells ameliorated stroke damage via several ways

involving immunomodulation and neuroprotection. In this study, we found that MSCs were unable to exert similar beneficial effect when transplanted in the chronic phase. This inability of MSCs in the treatment of chronic stroke could be attributed to the presence of unfavorable tissue environment. We genetically modified MSCs using adenovirus encoding several genes and transplanted in chronic stroke brain. Treatment with genetically modified MSCs significantly improved the behavioral and histological deficits when assessed with sensory-motor function tests and MRI respectively. Immunohistochemical analyses indicated that transplantation of those genetically modified stem cells rejuvenated the microenvironment of the damaged brain tissue and activated the quiescent endogenous neural stem cells in a transgenic mouse model where neural stem/progenitor cells can be detected as EGFP. We also obtained similar results in vitro studies indicating those cells exerted proangiogenic effects. Our genetically modified MSCs, satisfied the STEP3 guidelines suggested for preclinical studies by FDA and NIH (2014), might provide a potential therapeutic strategy for the treatment of chronic stroke.

Disclosures: **S. Marasini:** None. **S. Yoo:** None. **D. Chang:** None. **Y. Lee:** None. **S. Kim:** None. **H. Suh-kim:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.21/GG8

Topic: C.08.Stroke

Support: NSERC

CIHR

Title: Cell maturity plays a role in cell survival following transplantation into the stroke-injured brain

Authors: ***S. PAYNE**[1,2], P. ANANDAKUMARAN[2], M. COOKE[2], B. VARGA[6], C. MORSHEAD[3,4], A. NAGY[6], M. SHOICHET[1,2,5];
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Abstract: Worldwide 15 million people will suffer from a stroke each year, and up to two-thirds of survivors will experience life-long functional deficits. Despite the high prevalence of stroke, no clinical treatment exists that can replace lost cells and restore function. Recent regenerative

medicine strategies have focused on the transplantation of an exogenous population of cells to both provide local support to neurons of the brain and replace lost cells by integrating into the existing circuitry. While a promising strategy, most cells delivered to the brain fail to integrate into the host tissue, and usually perish. In order to address these issues, we investigated the use of a hydrogel delivery vehicle coupled with human induced pluripotent stem cell (iPSC)-derived cortical neural precursor cells (NPCs) of varying maturity for delivery into the rat stroke-injured brain. To increase cell survival following transplantation, our lab developed a hydrogel composed of hyaluronan and methylcellulose (HAMC) and demonstrated its ability to improve cell delivery and increase survival in models of spinal cord injury, retinal regeneration, and stroke. A population of human neuroepithelial cells (hNECs) was derived from iPSCs and then differentiated towards a neuronal lineage in vitro. Cell maturation over time was characterized using immunocytochemical staining for sox2, nestin, doublecortin, β-III tubulin, and MAP2. To determine the effect of cell maturity on survival in the stroke-injured brain, three time points of differentiation were chosen that displayed significant difference in neuronal marker expression in vitro: no differentiation (day 0), committed (day 16) and immature (day 32). First, the in vitro survival of these cells in HAMC was tested and it was determined that HAMC does not adversely affect cell survival. Next, rats were given a stroke in the motor cortex through injection of endothelin-1. One week following injury hNEC-derived cells were stereotactically injected into the injury site and, after 7 days, animals were sacrificed for immunohistochemical analysis. Both committed (d16) and undifferentiated (d0) neuronal cells had the highest number of cells present after 7 days, which was not due to differences in cell proliferation. The cell phenotype was also characterized for each group and it was found that the committed cell group contained the highest number of β-III tubulin positive cells. In ongoing studies, we are evaluating long-term cell survival and functional locomotor recovery.

Disclosures: **S. Payne:** None. **P. Anandakumaran:** None. **M. Cooke:** None. **B. Varga:** None. **C. Morshead:** None. **A. Nagy:** None. **M. Shoichet:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.22/GG9

Topic: C.09. Brain Injury and Trauma

Support: NJCBIR Fellowship Grant CBIR13FEL006

Title: Gpr161 regulates the adult neural stem cell neurogenesis post-traumatic injury

Authors: *M. ABABON[1], K. RONDEL[2], S. TENG[3], J. ALDER[3], P. MATTESON[2], J. MILLONIG[2,3];
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Abstract: Brain damage from injury, stroke and neurodegenerative diseases is irreversible, with no known treatment. Adult neural stem cells proliferate in response to injury including traumatic brain injury (TBI). Others have shown that inhibiting this response worsens the injury, while enhancing it improves cognitive recovery. Stimulating these stem cells is then a possible therapeutic strategy for brain injury. Understanding the molecular mechanisms regulating their neurogenesis will help develop therapeutics to aid repair after injury. We demonstrated that Gpr161, an orphan G protein coupled receptor (GPCR) is expressed in these adult CNS stem cell populations. Our previous work has shown that Gpr161 regulates retinoic acid (RA) signaling, which is required during adult neurogenesis in the hippocampal subgranular zone (SGZ). We then tested the hypothesis that Gpr161 regulates adult neurogenesis. First, *in vitro* lentiviral knockdown and overexpression of Gpr161 directly in adult-derived neurosphere cultures were performed. Gpr161 knockdown does not affect proliferation (EdU labeling) but increased the number of apoptotic cells (activated caspase-3 staining) ($p < 0.001$).Gpr161 overexpression resulted in a significant increase in EdU+ cells ($p < 0.001$) and decrease in activated caspase-3 + cells ($p < 0.05$).*In vivo,* lentiviral knockdown of Gpr161 in the SGZ decreased EdU ($p < 0.01$) and Dcx (neuronal differentiation marker) ($p = 0.06$) staining, and increased activated caspase-3 staining post-TBI. On the other hand, Gpr161 overexpression increased EdU+ ($p < 0.001$) and Dcx+ cells post-injury. To investigate the signaling pathway, we hypothesize that Gpr161 regulates adult neurogenesis through crosstalk between cAMP and RA signaling: Gpr161 activates cAMP and subsequent protein kinase A (PKA) phosphorylation of RA receptor alpha (RARa), increasing RARa nuclear translocation and transactivation activity, and increasing downstream RA signaling. Through immunocytochemistry for phosphorylated RARa (phosphoRARa) in our neurosphere cultures, we observed a decrease in nuclear staining of phosphoRARa in the knockdown ($p < 0.001$) and an increase in the overexpression ($p < 0.001$). Our results demonstrate that Gpr161, possibly through downstream cAMP and RA signaling, is both necessary and sufficient to regulate neurogenesis of adult neural stem cells in response to injury. Currently, 50-60% of available drugs target GPCRs, making them the most important family of pharmaceutical targets. Based on our study, Gpr161 may be a potential pharmaceutical target to treat brain damage.

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Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

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Program#/Poster#: 518.23/GG10

Topic: C.08.Stroke

Support: Funds from Ministry of Health, Labour and Welfare (Japan), Initiative for Accelerating Regulatory Science in Innovative Drug, Medical Device, and Regenerative Medicine (2012-2016)

Title: The next generation of autologous bone marrow stromal cell transplantation for stroke; MSC2.0

Authors: *H. SHICHINOHE, C. TAN, S. OH, T. ABUMIYA, N. NAKAYAMA, K. KAZUMATA, K. HOUKIN;
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Abstract: Background and purpose - Recent studies have elucidated that the bone marrow stromal cells (BMSCs) have therapeutic potential against stroke. Some clinical trials have been starting up in practice. We aimed to evolve the autologous BMSC transplantation for stroke into the next generation. **Materials and methods-** Human BMSCs were cultured with human platelet lysate (hPL) instead of fetal calf serum (FCS). They were labeled with superparamagnetic iron oxide (SPIO). Rat ischemic stroke models were made and $5x10^5$ cells were injected into the ipsilateral striatum stereotactically 7 days post-insult. Behavioral analysis, MRI for cell tracking, ^{18}F-FDG PET, and ^{123}I-Iomazenil SPECT were performed. The animals were sacrificed 5 to 8 weeks post-transplantation and histological analysis was performed. **Results-** There was no difference in the surface markers and cell proliferation between hPL and FCS. Although rotarod test showed that motor function deteriorated in rats suffered from permanent MCAo, BMSC-hPL transplantation enhanced recovery of the motor function, significantly. MRI demonstrated that SPIO-BMSCs aggressively migrated towards the lesion. Moreover, ^{18}F-FDG PET and ^{123}I-Iomazenil SPECT showed that BMSC transplantation promoted recovery of the glucose utilization and the binding potential of iomazenil in the peri-infarct area, respectively. Histological analysis supported the findings on MRI and showed the inclination for neural differentiation of donor cells. **Conclusion**- The hPL may be valuable and safe in expanding BMSCs. The application of bio-imaging techniques is also valuable for BMSC transplantation for stroke. Now we prepare the novel clinical trial against stroke, Research on advanced intervention using novel bone marrow stem cell (RAINBOW) study. The present results are translated into the optimal design of the trial.

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Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.24/GG11

Topic: C.08.Stroke

Title: Stem cell-based therapies for white matter stroke: neuronal vs glial progenitor cell treatment

Authors: *I. L. LLORENTE[1], D. R. MILLER[2], J. A. MAZZITELLI[2], J. CINKORNPUMIN[3], W. E. LOWRY[3], S. T. CARMICHAEL[2];
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Abstract: Subcortical white matter stroke (WMS) constitutes up to 30% of all strokes subtypes. There is no medical therapy that promotes recovery in this disease. Human induced pluripotent stem cells (IPSs) have shown benefit in some pre-clinical stroke models, but have never been tested in white matter stroke. In this study two stem/progenitor cell lines were used: human iPS-neuronal progenitor cells (NPCs) and human iPS-glial enriched progenitor cells (GEPs)-this cell line is differentiated from an iPS-NPC line into an immature glial cell. iPS-GEPs may be uniquely suited to repairing the brain after white matter stroke, as they produces immature glial cells that may both stimulate the injured cerebral white matter, and differentiate into replacement cells that have been lost in the stroke. We tested the effects of iPS-NPC and iPS-GEP transplantation in NSG mice in a subacute stage (7 days post-stroke) and performed tissue, MRI and behavioral outcome measures up to 6 months after transplantation. Both stem/progenitor cell lines presented a good engraftment and survival after 2 months. iPS-NPCs and iPS-GEPs behave very differently after transplantation. iPS-GEPs migrate widely in white matter after transplant, white the iPS-NPCs remained clustered in the infarct area. iPS-GEPs treatment stimulated endogenous neurogenesis, defined as the production of new neurons and glial cells, at a level 4 times than that seen with iPS-NPCs. Both iPS-NPC and iPS-GEP treatment led to remyelination in the stroke area 2 months post-transplantation (assessed by MBP staining). Motor testing showed iPS-GEPs uniquely improved motor recovery after stroke compared to the stroke-alone and stroke+iPS-NPCs. This white matter repair can be tracked in the living animal in real time with MRI, providing a biomarker for brain repair with this cell line in this disease. These findings suggest that transplanted iPS- progenitor cells could potentially replace lost cells and circuitry in ways that are unique to the subtype of the iPS- progenitor: GEP vs NPC.

Disclosures: I.L. Llorente: None. **D.R. Miller:** None. **J.A. Mazzitelli:** None. **J. Cinkornpumin:** None. **W.E. Lowry:** None. **S.T. Carmichael:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.25/GG12

Topic: C.08.Stroke

Support: NRF-2015M3A9B4067067

Title: To enhance the efficiency of delivery and engraftment of stem cells in a rat chronic stroke model

Authors: D.-R. CHO, D. CHANG, *H. K. SUH-KIM, G.-H. KIM, J. YOON, S.-S. KIM; Dept. of Anat., Ajou Univ, Sch. Med., Suwon, Korea, Republic of

Abstract: Mesenchymal stem cells (MSCs) have been reported to improve the recovery from ischemic stroke. However, MSCs have not been recognized clinically because their therapeutic effects were not demonstrated clearly, especially in chronic stroke patients. Moreover, routes and procedures for transplantation of MSCs have not been established. In our previous study, we showed that the transplantation of neurally-induced MSCs by introducing Neurogenin1 (MSC/Ngn1) dramatically improved the stroke outcome compared to the parental MSCs in an acute stroke model. In this study, we investigated whether MSC/Ngn1 cells were advantageous in a chronic stroke model compared to the parental MSCs. We also developed a transplantation procedure, which was less invasive than the intracranial injection while allowing an efficient engraftment of transplanted cells. We will discuss the results from experiments utilizing hyperosmotic agents to facilitate the infiltration of transplanted cells toward the infarcted area in a chronic stroke model. We propose that as a pre-clinical evidence, our results can be extended to the future clinical field for safe administration of functionally enhanced stem cells to the chronic stroke patients.

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Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: C.08.Stroke

Support: 16POST27790076

Title: Probing the stroke vasculome with endothelial progenitor cells

Authors: *S. A. ACOSTA, V. DE ALVARENGA GUEDES, J. LEE, Y. KANEKO, C. V. BORLONGAN;
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Abstract: Stroke is the number one cause of disability in the adult population and the fourth leading cause of death in the United States. Currently, the therapeutic interventions are limited with only one FDA- approved drug for ischemic stroke; namely tissue plasminogen activator or tPA. Recent novel findings revealed that cerebral endothelium can secrete molecules that may regulate disease processes following ischemic stroke; namely inflammation-associated vasculome. In the present in vitro study, we evaluated the therapeutic effect of endothelial progenitor cells on the inflammation-associated stroke vasculome. Briefly, human endothelial cells (HEN6) were prepared and grown for 10 days. qRT-PCR analysis of the expression of specific stroke vasculome genes revealed that under ambient condition, basal levels of BRM, IκB, foxf1, and ITIH-5 could be detected (compared to non-endothelial cells NT2N acting as control), but following oxygen glucose deprivation (OGD), there were significant elevations in all 4 inflammation-associated stroke vasculome genes (P's<0.05 vs. respective level of each gene in ambient condition). Interestingly, co-culture of HEN6 with human EPCs (1:1 ratio) during the OGD treatment significantly blocked the elevations of BRM, IκB, and foxf1 (P's<0.05 vs. respective level of each gene in OGD condition), but not ITIH-5 (P>0.05). Next, employing the knockdown/antisense technology, silencing the inflammation-associated stroke vasculome gene, IκB, as opposed to scrambled knockdown, blocked the EPC-mediated protection of HEN6 against OGD (P's<0.0 vs. OGD or OGD+ IκB knockdown). These results implicate a close interaction between EPCs and stroke vasculome, which appear to be therapeutically modulating the inflammatory insult after stroke.

Disclosures: S.A. Acosta: None. **V. De Alvarenga Guedes:** None. **J. Lee:** None. **Y. Kaneko:** None. **C.V. Borlongan:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

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Topic: C.08.Stroke

Support: the Swedish Medical Research Council-VR 2008-2286

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china scholarship council (csc)

Title: What role do immune cells play in preterm newborn brain injury?

Authors: *X. ZHANG[1,2], D. JABIN[1], K. ZHOU[3], A. NAZMI[1], A.-M. ALBERTSSON[1], K. SOBOTKA[1], C. ZHU[3], H. HAGBERG[1,4,5], C. MALLARD[1], X. WANG[1];
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Abstract: Background/Objective Infants with sepsis have increased incidence of cerebral palsy, white-matter injury, and brain abnormalities, and these are especially common in low birthweight newborn infants. It is now accepted that immune cells derived from the systemic circulation are a key feature of many diseases of the central nervous system. The aim of this study was to investigate whether or not immune cells contribute to the development of preterm brain injury using a mouse model of sepsis. **Methods** C57BL/6J (wild-type), T-cell receptor delta knockout (deltaTCRKO), and T-cell receptor beta knockout (betaTCRKO) mice were subcutaneously administrated sterile saline or lipopolysaccharide (LPS) (5 mg/kg) at postnatal day (PND) 2. Pups were sacrificed at PND12, and their brains were collected. Brain sections were stained for myelin basic protein, which is the brain white-matter marker. Motor function and anxiety were evaluated at PND 26-30, which is comparable to human teenage years, using Digi Gait analysis and an elevated plus maze. **Results** LPS causes the loss of brain white matter volume in wild-type mice and tissue loss in the betaTCRKO mice compared with their saline-treated groups. However, deltaTCRKO mice did not have any white-matter tissue loss after LPS compared to the controls. The different treatments and genotypes had no effect on anxiety behavior in the mice as determined with the elevated plus maze. Digi Gait analysis showed that there were increases in stance, stride, and stride length and a decrease in stride frequency after LPS treatment in the wild-type and betaTCRKO mice, but no such effect was observed in the deltaTCRKO mice. **Conclusions** 1. LPS causes sepsis-induced damage to the white matter in the

brain. 2. Gamma/delta T cells, but not alpha/beta T cells, contribute to sepsis-induced brain injury and mild motor abnormalities in early life of mice.

Disclosures: **X. Zhang:** None. **D. Jabin:** None. **K. Zhou:** None. **A. Nazmi:** None. **A. Albertsson:** None. **K. Sobotka:** None. **C. Zhu:** None. **H. Hagberg:** None. **C. Mallard:** None. **X. Wang:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.28/HH1

Topic: C.08.Stroke

Support: NIH R01HD061363

Title: Hyperactive effect of BrdU on an endothelin 1 induced stroke model of rat

Authors: ***H. H. CHAN**[1,2], J. COOPERRIDER[2], H. PARK[2], J. T. GALE[2], K. B. BAKER[2], A. G. MACHADO[2];
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Abstract: Introduction:
5-bromo-2'-dexoyuridine (BrdU) is often used to label newly-divided cells in the context of experimental research. However, several studies suggest that BrdU may produce unwanted side effects; including changes in animal behavior that might impact behavioral assays and healthy cell function in postnatal animals. In this study, we investigated the effect of BrdU on locomotor behavior as well as GABAergic and glutamatergic neurotransmission in a rodent model of ischemic stroke.
Method:
Ischemic strokes were induced in adult rats using 6 injections of 800pmol endothelin 1 into the left motor cortex. 50mg/kg BrdU was intraperitoneally injected over 5 days beginning 2 weeks post-stroke, while stroke control animals received only saline. Locomotor activity was evaluated by videotaping the rats in their home cages for 30 min, beginning one hour after BrdU injection. Rats were sacrificed 6 weeks after stroke induction. The glutamatergic and GABAergic tract integrity in the perilesional cortex and striatum was analyzed by immunohistochemistry.
Result:
BrdU-treated rats showed increase locomotor activity than control animals ($p<0.05$). Furthermore, there was a greater reduction of GABAergic signal in the perilesional cortex and striatum of BrdU-treated animals, while the magnitude of the observed reduction in

glutamatergic signal did not differ between BrdU-treated and untreated animals.
Conclusion:
These findings suggest that BrdU induces a hyperlocomotor effect in rats, possibly due to a selective degeneration of GABAergic innervation in both perilesional motor cortex and striatum.

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Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

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Support: National Institute of Neurological Disorders and Stroke RO1 NS083078 (J.C.) NS088656 (M.C.) and 41NS064708 (J.C.)

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Title: Comparison of the therapeutic effects of BMSCs derived from normal or T1DM rats and the role of T1DM-miR145 in the treatment of ischemic stroke

Authors: ***A. ZACHAREK**[1], C. CUI[3,2], M. CHOPP[2], P. VENKAT[2], T. YAN[2], R. NING[2], P. YU[2], J. CHEN[2];
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Abstract: Background: Treatment of stroke with bone-marrow-stromal cells (BMSCs) derived from normal rats (Nor-BMSCs) at 24h after stroke onset improves functional recovery in non-DM rats, but not in type-one DM (T1DM) rats. In the present study, we tested the therapeutic effects and mechanisms of action when treating T1DM stroke with BMSCs derived from DM rats (DM-BMSCs) or Nor-BMSCs. The potential role of microRNA-145 (miR-145) in mediating the enhanced DM-BMSC treatment induced benefits was also investigated.
Methods: T1DM rats underwent 2 hours of middle cerebral artery occlusion (MCAo) and were treated 24 hours later with (n=8/group, 5×10^6 cells, i.v.) 1) Phosphate Buffered Saline (PBS); 2)

Nor-BMSCs; 3) DM-BMSCs; 4) DM-BMSCs with miR-145 over-expression (miR-145+/+DM-BMSCs); 5) Nor-BMSCs with miR-145 knockdown (miR-145-/-Nor-BMSC). Evaluation of functional outcome, vascular and white matter remodeling and microRNA expression, and in-vitro studies were performed.
Results: Compared with Nor-BMSCs or PBS control, treatment of stroke in T1DM rats with DM-BMSCs significantly improved functional outcome, decreased serum miR-145 expression, as well as increased expression of miR-145 target genes, adenosine triphosphate-binding cassette transporter 1 (ABCA1) and insulin-like growth factor 1 receptor (IGFR1). DM-BMSCs increased vascular remodeling, identified by cerebral vascular density and cerebral artery density, increased Bielschowsky silver (axon marker) and Luxol fast blue (myelin marker) expression in the ischemic border zone (IBZ) compared to PBS treated T1DM-MCAo rats. We also found that DM-BMSCs exhibited decreased miR-145 expression and increased BMSC survival compared with Nor-BMSCs. DM-BMSCs conditioned medium significantly decrease smooth muscle cell (SMC) and oligodendrocyte (OL) cell death compared to Nor-BMSCs conditioned medium in vitro. In vivo, miR-145+/+DM-BMSCs significantly increased blood serum miR-145 expression and decreased brain ABCA1 and IGFR1 expression as well as attenuated DM-BMSCs induced neurorestorative effects in T1DM-MCAo rats**.** miR-145-/-Nor-BMSC treatment of stroke in T1DM rats significantly improved functional outcome compared to PBS or Nor-BMSCs treated T1DM-MCAo rats, respectively.
Conclusion: Treatment of stroke in T1-DM rats with DM-BMSCs significantly increased neurovascular remodeling and improved neurological recovery compared with Nor-BMSC and PBS treated T1-DM rats. DM-BMSCs exhibit decreased miR-145 expression compared to Nor-BMSCs. The miR-145/ABCA1/IGFR1 pathway may contribute to the enhanced functional and neurorestorative effects of DM-BMSCs in T1DM stroke rats.

Disclosures: **A. Zacharek:** None. **C. Cui:** None. **M. Chopp:** None. **P. Venkat:** None. **T. Yan:** None. **R. Ning:** None. **P. Yu:** None. **J. Chen:** None.

Poster

518. Cellular Mechanisms and Stroke Injury: Macrophage-Microglia and Stem Cells

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 518.30/HH3

Topic: C.08.Stroke

Support: Axencia Galega de Innovación (Xunta de Galicia)

Instituto de Salud Carlos III (PI13/00292; PI14/01879)

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Promoting Active Ageing: Functional Nanostructures For Alzheimer's Disease At Ultra-Early Stages" (Pana_686009) is a Research and Innovation Project, funded within the EU Horizon 2020 Programme

Title: Mesenchymal stem cell MRI tracking of intraarterial and intravenous therapy in cerebral ischemia.

Authors: *T. SOBRINO[1], B. ARGIBAY[2], E. LOPEZ-ARIAS[2], U. HIMMELREICH[4], C. CORREA-PAZ[2], I. LOPEZ-LOUREIRO[3], M. PEREZ-MATO[3], R. IGLESIAS-REY[3], J. RIVAS[5], J. CASTILLO[3], F. CAMPOS[3];
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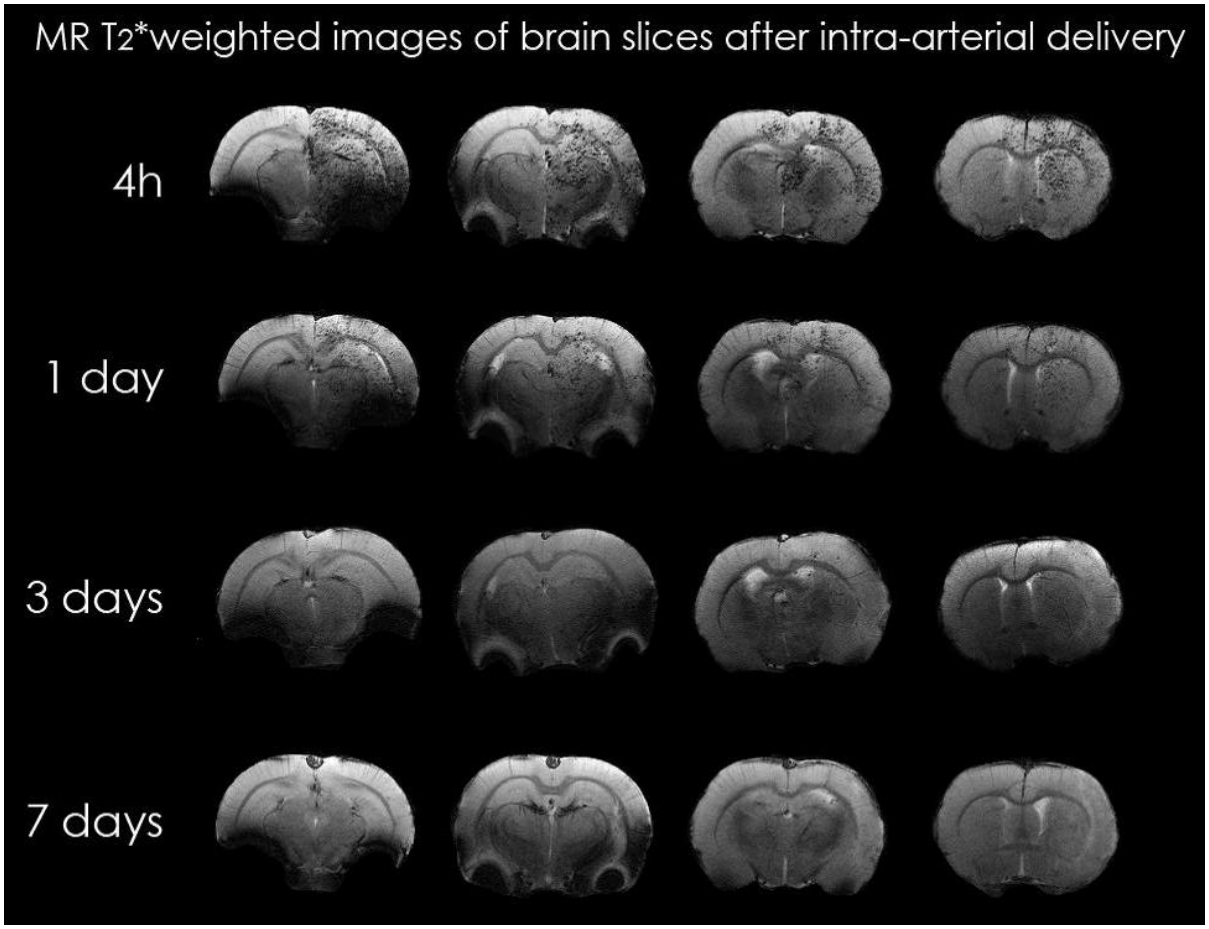
Abstract: Rationale: Mesenchymal stem cells (MSCs) represent a promising clinical therapy for ischemic stroke; however, critical parameters, such as the most effective administration route, remain unclear. Intravenous (i.v.) and intraarterial (i.a.) delivery routes, most frequently used for cell administration, have yielded varied outcomes across studies, presumably due to the unknown distribution of MSCs. In this regard, cell tagging by contrast agents (CAs), coupled with magnetic resonance imaging (MRI) analysis, represents a powerful technique to accomplish this challenge.
Objective: In this study, dextran-coated superparamagnetic nanoparticles (D-MNPs) were synthesized as CAs and validated for cell tracking in MRI. Secondly, the protocol for i.a. cell administration was optimized to improve efficiency and safety. The cellular biodistribution of MSCs after i.a. and i.v. administration was also investigated. Finally, the therapeutic effects of MSCs administered via both routes were compared in an animal model of ischemic stroke.
Methods and Results: MSCs labeled with D-MNPs were detected in the brain by magnetic resonance (MR) T_2*weighted images after i.a. injection (**Figure 1**). Transmission electron microscopy (TEM) showed cells not only in small cerebral vessels, but also in the brain parenchyma. However, after i.v. administration, cells were localized exclusively to the lungs. I.A. administration was not found to be more advantageous than i.v. administration, as cerebral edema and cerebral lesions increased following i.a. delivery.

Conclusions: i.a. administration represents an efficient strategy to engraft MSCs in the brain after an ischemic stroke; however, this method of delivery presents the risk of cerebral lesions, without greater benefits than i.v. after ischemic stroke.
Figure 1 Legend: MR T2* weighted images of one ischemic animal injected (IA) with 0.25x106 D-MNPs labeled MSCs at different time-points.



Disclosures: **T. Sobrino:** None. **B. Argibay:** None. **E. Lopez-Arias:** None. **U. Himmelreich:** None. **C. Correa-Paz:** None. **I. Lopez-Loureiro:** None. **M. Perez-Mato:** None. **R. Iglesias-Rey:** None. **J. Rivas:** None. **J. Castillo:** None. **F. Campos:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.01/HH4

Topic: C.08.Stroke

Title: The NINDS Repository: a large public collection of biomaterials for neurological disease research.

Authors: C. A. PEREZ[1], *S. G. HEIL[1], J. SANTANA[1], A. GREEN[1], A. AMBERSON[1], R. ZHANG[2];
[1]NINDS Repository, Coriell Inst. For Med. Res., Camden, NJ; [2]NIH-NINDS, Bethesda, MD

Abstract: Neurological disorders are a serious health concern that presents massive challenges to healthcare systems globally and their multifactorial pathological mechanisms are not completely understood. The National Institute of Neurological Disorders and Stroke (NINDS) - part of the USA National Institutes of Health (NIH)- sponsors the NINDS Repository which was established in 2002 at the Coriell Institute for Medical Research with the mission of supporting the identification of the genetic risks and causes for neurological disorders. The NINDS Repository collects blood samples as well as de-identified clinical data from a diverse patient population diagnosed with cerebrovascular diseases, Parkinsonism, motor neuron diseases, epilepsy, Tourette syndrome, Dystonia, and neurologically normal controls. The collection features patient-derived DNA and cell lines including many samples annotated with well-defined mutations. Since the NINDS Repository inception, biomaterials and clinical data from more than 46,000 individuals have been received. More than 38,000 unique samples are available through an online catalog at http://catalog.coriell.org/1/NINDS and since 2003 more than 40,000 samples have been distributed to investigators worldwide.The NINDS Repository aims to standardize the collection and processing across all samples while protecting patient safety and privacy. In an effort to ensure the quality of these valuable biological resources, the NINDS Repository has established well validated standard operating procedures (SOPs) for the collection, reception, processing, storage, and worldwide distribution of biological specimens. These SOPs include rigorous quality control assessments for each sample type. The NINDS Repository aims to provide rapid feedback to sample submitters regarding sample integrity and appearance at the time of receipt and status after completion of all required in-house processing. Essential to all this is a customized secure and highly user-integrated biobanking laboratory information management system, including sample-data association by cross-referencing with other NIH resources such as dbGaP.The development of such a centralized collection of human biospecimens and their associated de-identified clinical data allows the NINDS Repository to provide a vital resource for research designed to discover and validate genetic and molecular biomarkers relevant for the study and treatment of neurological disorders prevalent in our society.

Disclosures: **C.A. Perez:** None. **S.G. Heil:** None. **J. Santana:** None. **A. Green:** None. **A. Amberson:** None. **R. Zhang:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.02/HH5

Topic: C.08.Stroke

Support: Casa Colina Foundation

Title: The effect of hyperbaric oxygen therapy on blood biomarkers and functional impairments following an ischemic stroke

Authors: *E. ROSARIO[1], S. KAPLAN[2], S. ROSENBERG[2];
[1]Res. Inst., Casa Colina Hosp. and Centers For Healthcare, Pomona, CA; [2]Casa Colina Hosp. and Centers for Healthcare, Pomona, CA

Abstract: Objective: While research suggests a benefit of hyperbaric oxygen therapy (HBOT) for neurologic injury, controlled clinical trials have not been able to clearly define the benefits. The objective of this study was to investigate the mechanistic and functional effects of hyperbaric oxygen therapy (HBOT) in the treatment of ischemic stroke.
Methods: Subjects were included in this study if they had suffered an ischemic stroke approximately 12-month ago and exhibited some functional impairments. Using a within subject design a baseline for current functional abilities was established over a 3-month period for all subjects. Each subject then received two 4-week periods of HBOT for a total of 40, 90-minute treatments over a 12-week period. Subjects completed a battery of assessments including cognitive, physical, speech, and quality of life measures, and had blood drawn for biochemical analysis of biomarker expression levels six times over the 9-month total duration of the study.
Results: We found improvements in cognition and executive function as well as physical abilities specifically, improved gait. Participants reported improved sleep and quality of life following HBOT treatment. We also saw changes in serum levels of biomarkers for inflammation and neural recovery. In the functional domains where improvement was observed following HBOT treatment, the improvements were maintained up to 3 months following the last treatment. However, the physiological biomarkers showed a pattern of more transient changes following HBOT treatment.
Conclusions: Findings from this study support the use of HBOT as a potential intervention following stroke, even after patients have plateaued in recovery. In addition these finding suggest the potential value of monitoring blood based inflammatory and neural markers as indicators of a physiological response to treatment.

Disclosures: E. Rosario: None. **S. Kaplan:** None. **S. Rosenberg:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.03/HH6

Topic: C.09. Brain Injury and Trauma

Support: NIH RO1 NS050465

NIH R01 DK104363

American Heart Association/13SDGETRGO0032

Title: System biology reveals genomic-wide alterations linking brain trauma with neurological disorders

Authors: *F. GOMEZ-PINILLA[1,2,3], Y. MENG[1], X. YANG[1];
[1]Integrative Biol. and Physiol., [2]Brain Injury Res. Ctr., [3]Dept. Neurosurg., UCLA, Los Angeles, CA

Abstract: The complexity of the pathology of traumatic brain injury (TBI) involving multiple components poses pressing challenges for development of effective methods to diagnose the pathology and to develop therapeutics. We carried out a systems biology study using next generation sequencing and integrative genomics analyses to determine how TBI affects networks of genes that could characterize main events in the pathology, and how these signatures could serve as biomarkers of mild TBI (mTBI). We found that mTBI promoted reprogramming of important aspects of gene regulation (DNA methylation, transcript abundance, alternative splicing, and organization of genes in networks), which have the potential to alter the course of brain homeostasis and disease. We also analyzed leukocytes to trace homologies between central and peripheral events, and found homology between hippocampus and blood at gene-, pathway-, and network levels. Transcriptomic signatures in the hippocampus overlap with those in leukocytes, and that these signatures correspond to curated functional categories related to vascularity, cell integrity, and immune response. mTBI also elicited select methylomic changes in hippocampus and leukocytes that colocalized with the respective transcriptomic signature genes. We also found that mTBI promotes specific changes in the organization of genes in networks, under the regulatory control of key driver genes such as *Anxa2* and *Ogn*. Gene networks identified in our rodent model of mTBI overlapped with candidate causal genes in human gene-wide association studies (GWAS) for several neurodegenerative diseases such as Alzheimer's disease, bipolar disorder, autism, cognitive performance, PTSD, and psychiatric disorders, which are proposed long-term sequel of mTBI. The overall results support the potential of our novel unbiased strategy to diagnose and to treat mTBI. In addition, our approach has the promise to get insightful information to elucidate one of the most intriguing aspects of

mTBI, which is why many patients become vulnerable to a range of neurological disorders such as CTE, Alzheimer's, and psychiatric.

Disclosures: **F. Gomez-Pinilla:** None. **Y. Meng:** None. **X. Yang:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.04/HH7

Topic: C.09. Brain Injury and Trauma

Support: NJCBIR15IRG014

Title: Cardiovascular changes in the Wistar rats after the traumatic brain injury.

Authors: *V. C. CHITRAVANSHI[1], H. N. SAPRU[2];
[1]Neurolog. Surgery, Rutgers The State Univ. of New Jersey, Newark, NJ; [2]Neurosurg., Rutgers-NJMS, Rutgers State Univ. of NJ, Newark, NJ

Abstract: Cardiovascular complications often accompany traumatic brain injury (TBI) and their negative impact on the recovery of these patients is well documented. There are no reports in the literature regarding the alterations in brain areas controlling cardiovascular function in TBI. In this study we investigated the effects of mild (0.1-1 atmospheres), moderate (1.5-2.2 atmospheres) and severe (2.8-3.6 atmospheres) traumatic brain injury (TBI) on systemic blood pressure (BP), and heart rate (HR). Alterations induced by TBI in the responses elicited by chemical stimulation of the medial subnucleus of nucleus tractus solitarius (mNTS), caudal ventrolateral medullary depressor area (CVLM) and rostral ventrolateral medullary pressor area (RVLM) were also studied. The experiments were done in adult, male Wistar rats, weighing 325-350 gm, anesthetized with either isoflurane or urethane. Fluid percussion was applied to induce TBI. Application of severe, moderate and mild TBI resulted in 100%, 33% and 10% mortality, respectively. The rats were unconscious for 12.4 ± 3.9 min and 4.6 ± 1.6 min after application of moderate and mild TBI, respectively. Application of moderate and mild TBI resulted in apnea for 8.2 ± 2.8 and 1.8 ± 1.1 sec, respectively. The decreases in mean arterial pressure (MAP) elicited by application of moderate TBI were 29.8 ± 3.4, 14.2 ± 5.4, 29.1 ± 7.9, 42.6 ± 7.1, 43.3 ± 3.4 and 51.4 ± 10.2 (mmHg) after 5, 30, 60, 120, 240 and 360 min of injury, respectively. The decreases in HR elicited by moderate TBI were 30.6 ± 10.4, 72.4 ± 28.6, 84.3 ± 29.1, 98.1 ± 32.7, 103 ± 31.6 and 134.6 ± 54 (bpm), respectively, at the same time points. The decreases in MAP elicited by mild TBI were 24.8 ± 3.4, 9.6 ± 5.7, 20 ± 4.8, 38 ± 6.3, 38 ± 8.6 and 49.3 ± 5.1 (mmHg) at the same time points. HR decreases elicited by mild TBI at corresponding time points

were 25.8 ± 10.9, 65.6 ± 28.8, 80.8 ± 28.8, 79.3 ± 30.5, 85.3 ± 30.2 and 92.8 ± 29.6 (bpm), respectively. These results indicate that both moderate and mild TBI elicit decreases in MAP and HR.

Disclosures: V.C. Chitravanshi: None. **H.N. Sapru:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.05/HH8

Topic: C.09. Brain Injury and Trauma

Support: NIH R01NS089051

VA RR&D#B6761R

Title: Changes of hippocampal autophagy and lysosome function after traumatic brain injury

Authors: E. LI[1], Y. YIN[1,2], G. SUN[1], I. ATTARWALA[1], G. BEGUM[1], H. YAN[3,4], K. KISELYOV[5], E. DIXON[3], *D. SUN[1,4];
[1]Dept. of Neurol., Univ. of Pittsburgh Med. Sch., Pittsburgh, PA; [2]Neurol., the Second Hosp. of Dalian Med. Univ., Dalian, China; [3]Neurosurg., Brain Trauma Res. Center, Univ. of Pittsburgh, Pittsburgh, PA; [4]Veterans Affairs Pittsburgh Hlth. Care Syst., Geriatric Research, Education, and Clin. Ctr., Pittsburgh, PA; [5]Biol. Sci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Traumatic brain injury (TBI) triggers ER stress and toxic protein accumulation (unfolded, and misfolded, or protein aggregates). Impaired autophagy flux and lysosomal function decrease autophagic clearance of damaged organelles and toxic macromolecules and contribute to neuronal death after TBI. We previously reported that post-injury administration of docosahexaenoic acid (DHA) reduces ER stress and improves neurological function after TBI. In this study, we investigated whether administration of DHA improves autophagy flux in the hippocampus after TBI. TBI was induced by cortical contusion injury in Sprague Dawley rats, which received DHA (16mg/kg in DMSO) or vehicle DMSO (1 ml/kg) daily interperitonial administration with an initial dose at 15 minutes after the injury. Changes of autophagy and lysosomal function in hippocampal tissues at 3 days after TBI were evaluated by Western blot analysis. First, TBI triggered significant accumulation of ubiquinated protein (4.1 ± 1.2 fold, $p < 0.05$) and SQSTM1/p62 (sequestosome 1) expression (19.8 ± 9.1 fold, $p < 0.05$). In addition, there was a 3.8 ± 1.2 fold increase ($p < 0.05$) in LAMP1 (lysosomal-associated membrane protein 1) expression but significant decrease in cathepsin D protein level (0.41 ± 0.15 fold; $p <$

0.01) in the injured hippocampus. In contrast, no differences between the contralateral and ipsilateral cathepsin D protein expression ($p > 0.05$) were detected in the DHA-treated hippocampus. Moreover, there is a trend that post-injury DHA administration maintains a higher hippocampal LAMP1 and SQSTM1/p62 expression. Taken together, our study suggests that TBI activates autophagy but decreased hippocampal autophagy flux and lysosomal functions. Post-injury DHA administration reduces ER stress and improves lysosomal function, which could collectively facilitate tissue repair, demonstrating its therapeutic potential to ameliorate TBI-induced injury.

Disclosures: **E. Li:** None. **Y. Yin:** None. **G. Sun:** None. **I. Attarwala:** None. **G. Begum:** None. **H. Yan:** None. **K. Kiselyov:** None. **E. Dixon:** None. **D. Sun:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

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Program#/Poster#: 519.06/HH9

Topic: C.09. Brain Injury and Trauma

Support: NeuroSurgical Research Foundation

University of Adelaide Kick Start Grant

Title: Long-term functional outcomes are associated with synaptic and axonal loss in the hippocampus following traumatic brain injury: Relationship to neurodegenerative disease.

Authors: *L. E. COLLINS, A. ARULSAMY, J. TENG, C. VAN DEN HEUVEL, F. CORRIGAN;
Univ. of Adelaide, Adelaide, Australia

Abstract: Background: Traumatic brain injury (TBI) is a leading cause of disability and death worldwide, affecting as many as 54-60 million people annually. While much previous research has focused on impairment immediately following injury, TBI is also recognized as a significant risk factor for the development of dementia and Alzheimer's disease. Alarmingly, the risk of developing dementia has been shown to be increased at least 2-fold and 4-fold in patients who have suffered moderate and severe TBI, respectively. Despite growing awareness of the relationship between TBI and dementia, however, the brain mechanisms that may account for this relationship are currently unknown. Recently, it has been proposed that structural damage caused by axonal injury in TBI may interact with neuroinflammation in the pathogenesis of dementia.

Objective: The objective of the current study was to investigate the persistent post-TBI neuroinflammatory response and how it mediates the development of neurodegeneration and functional impairment.
Methods and Materials: Sprague-Dawley rats (n= 10/group) underwent either sham surgery or an experimental model of moderate-severe TBI. At 1-month and 3-months post-injury, rats completed a functional battery testing motor function, depressive-like behaviour, anxiety and cognition. Following testing, Western blot analysis was conducted to assess the protein expression of a range of inflammatory, neurodegenerative and oxidative stress markers.
Results: At both 1 and 3-months post injury, depressive-like behavior was significantly increased in TBI animals compared to shams. Additionally, at 3-months post-injury, TBI animals exhibited significant cognitive impairment. While markers of synaptic integrity and astrocyte expression both appeared to be significantly decreased in the frontal lobe at 1-month post-injury, this effect was no longer seen by 3-months post injury. Interestingly, while hippocampal tissue appeared normal, with the exception of an elevation of astrocyte expression, at 1-month post-injury, by 3-months post-injury, both synaptic and axonal integrity were reduced, and expression of the apoptotic marker active caspase-3 was significantly increased.
Conclusion: While transient changes to synaptic integrity of the frontal cortex are observed acutely following TBI, persistent neurodegenerative changes within the hippocampus do not appear until the chronic phase of injury, concomitant with the emergence of cognitive decline. Understanding specific brain mechanisms that may link TBI to dementia is a critical first step in developing novel treatment strategies for long-term complications of TBI.

Disclosures: L.E. Collins: None. **A. Arulsamy:** None. **J. Teng:** None. **C. Van den Heuvel:** None. **F. Corrigan:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.07/HH10

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant 1R01AG042890 to GT

Title: Decreased synaptic insulin responsiveness in the hippocampus of traumatic brain injured rats

Authors: *W. FRANKLIN[1], G. TAGLIALATELA[2];
[2]Mitchell Ctr. for Neurodegenerative Diseases, Dept. of Neurol., [1]UTMB At Galveston, Galveston, TX

Abstract: Alterations of insulin signaling in neurons have been linked to many disorders including Alzheimer's disease (AD). Decreased insulin signaling increases synaptic sensitivity to amyloid beta (Aβ), a toxic protein in AD, thus contributing to the cognitive decline that characterizes this neurodegenerative disorder. Traumatic brain injury (TBI) is a risk factor for later development of AD, although the mechanisms contributing to this increased risk are unknown. To determine whether decreased insulin responsiveness in TBI animals is playing a role in the synaptic vulnerability to AD pathology, we developed a method for studying the insulin responsiveness at the synaptic level. We isolated functional synaptosomes from frozen rodent brain tissue and exposed them to insulin in the presence of ATP to detect insulin receptor (IR) phosphorylation (activation). Using this method coupled to Western blot analysis, we were able to detect insulin-driven phosphorylation of the synaptic IR, proportionate to the known extent of insulin responsiveness/resistance of the input samples. After optimizing this method, we analyzed synaptosomal insulin responsiveness in the hippocampi of SHAM and TBI animals that underwent a lateral fluid percussion injury. Our results indicate that there is decreased insulin responsiveness 2 days post injury that is exacerbated by 7 days, primarily in the hippocampus of the injured hemisphere. In conclusion, we were able to detect acute dysregulation in synaptic insulin responsiveness in the brain of rats after traumatic brain injury warranting further experiments to look at chronic alterations and downstream elements. These initial results further suggest that synaptic insulin resistance may occur as a consequence of TBI, a condition that is know to sensitize synapses to the dysfunctional impact of Aβ oligomeric species and thus increase susceptibility to AD-related cognitive decline.

Disclosures: W. Franklin: None. **G. Taglialatela:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.08/HH11

Topic: C.09. Brain Injury and Trauma

Title: A novel quantitative multianalyte immunoassay to detect peripheral blood biomarkers following traumatic brain injury

Authors: M. ANDERSON, *G. HICKEY, I. O'BRIEN, P. YOUNGE, L. LEONG;
Bio-Techne Inc, Minneapolis, MN

Abstract: Traumatic Brain Injury (TBI) is a worldwide health concern that affects about 10 million people globally every year. The majority of TBI cases are classified as mild, and many of these individuals do not exhibit any visible physical signs of head trauma, making it difficult to

identify TBI. Early detection of TBI is crucial in order to limit the primary and secondary brain damage that may occur following head trauma. Thus, there is a need for the early detection and measurement of circulating biomarkers that reflect a traumatized state. Research has been conducted to identify potential markers of TBI in cerebral spinal fluid (CSF). However, because of the invasiveness in obtaining CSF samples, CNS-specific markers of TBI in peripheral blood need to be discovered. Unfortunately, most standard immunoassays do not have the sensitivity to detect markers of neural cell damage and neuroinflammation in the peripheral blood.
The Simple Plex™ (ProteinSimple) assay is a novel, quantitative, multianalyte immunoassay platform that delivers high precision and accuracy while using less than 25 μL of sample. This highly sensitive platform measures up to four analytes simultaneously from a single, small sample with results generated in just over an hour. The microfluidic-based system allows parallel single analyte quantification and reduces the non-specific antibody interactions often observed in other traditional multiplex platforms. The whole process is automated within a single cartridge, thus removing potential user variability. The Simple Plex™ assay was employed to look at several proposed TBI markers in serum samples from individuals diagnosed with TBI. Our study suggests that this platform may represent a highly efficient method for detecting and quantifying markers of neural cell damage and neuroinflammation in the peripheral blood following TBI.

Disclosures: **M. Anderson:** None. **G. Hickey:** None. **I. O'Brien:** None. **P. Younge:** None. **L. Leong:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

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Program#/Poster#: 519.09/HH12

Topic: C.09. Brain Injury and Trauma

Support: DOD Grant W81XWH-13-1-0384

R01ES024233

R01AG037481

R01AG037919

K01AG044490

Title: Integrated genomic approaches identify changes in interconnected networks and major pathways following traumatic brain injury in young and aged APOE3 and APOE4 mice

Authors: *E. L. CASTRANIO[1], A. MOUNIER[1], C. M. WOLFE[1], J. SCHUG[2], N. F. FITZ[1], R. KOLDAMOVA[1], I. LEFTEROV[1];
[1]Envrn. and Occup. Hlth., Univ. of Pittsburgh, Pittsburgh, PA; [2]Dept. of Genet., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability in army personnel and general population. Expression of human Apolipoprotein E (APOE) modulates inflammatory response in an isoform specific manner, with APOE4 isoform eliciting a stronger pro-inflammatory response, suggesting a possible mechanism for worse outcome following TBI and in AD. We hypothesized that inheritance of APOE4 would lead to worse cognitive outcome and higher inflammatory gene expression following TBI with age as an additive factor for worsened outcome. This study uses mice with targeted replacement of endogenous *Apoe* by human cDNA for expression of APOE3 (E3) and APOE4 (E4). Young adult, 3 month old, and aged, 9 month old mice, received either a controlled cortical impact (CCI) brain injury or a craniotomy in the left hemisphere. In young mice, TBI groups performed significantly worse than sham ($p<0.05$) in Morris Water Maze (MWM), regardless of genotype. In aged mice, performance in MWM was significantly different between sham and TBI groups in E3, but not in E4 mice. To investigate the molecular mechanisms underlying this effect, we performed genome-wide differential gene expression analysis using RNA-seq on libraries generated from cortices and hippocampal tissues of mice from all groups and applied weighted gene-coexpression network analyses (WGCNA). In the co-expression networks, we were most interested in WGCNA modules that were significantly differentially expressed between groups, such as sham vs TBI, E3 vs E4, or young vs aged. The module most significantly related to TBI is brown, with a consensus for gene up-regulation at $p = 1e\text{-}10$. The annotation in the module demonstrated genes strongly enriched for GO terms “Immune response” and “inflammatory response”. Hub genes are the most highly connected genes within the module network, and of interest were Tyrobp, Clu and Axl. A representation of gene connectivity for the brown module, was built using Cytoscape and included *Trem2, Cx3cr1* and *Abca1*. We validated these genes using RT-QPCR expression assays, and confirmed that TBI caused significant upregulation of their gene expression. The results of our study indicate that TBI worsens cognitive deficits in an age specific manner, with E4 mice displaying a basal cognitive deficit that becomes more apparent in aged mice. Additionally, TBI increases expression of inflammatory genes related to a microglial response to injury.

Disclosures: E.L. Castranio: None. **A. Mounier:** None. **C.M. Wolfe:** None. **J. Schug:** None. **N.F. Fitz:** None. **R. Koldamova:** None. **I. Lefterov:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.10/HH13

Topic: C.08.Stroke

Support: PHS Grant MH104800

New Jersey Health Foundation Inc.

Title: Acute ischemic stroke was not associated with circulating interleukin-1 and tumor necrosis factor, but did result in post-stroke depression

Authors: S. NORTON[1], R. GLASS[1], S. MANN[1], M. MOCCIO[2], A. ZIMMERMAN[3], N. FIEDLER[4], R. CONTRADA[1], M. MENZA[3], H. LEVENTHAL[1], J. MCKINNEY[6], *A. W. KUSNECOV[5];
[1]Psychology, [2]Neurol., [3]Psychiatry, [4]Envrn. and Occup. Med., Rutgers Univ., New Brunswick, NJ; [5]Psychology, Rutgers Univ., Piscataway, NJ; [6]Neurol., New Hanover Regional Med. Ctr., Wilmington, NC

Abstract: Acute ischemic stroke (AIS) has been associated with elevations in circulating inflammatory cytokines. In addition, a subset of AIS patients can develop clinically significant depression (post-stroke depression [PSD]), which can compromise recovery and increase recurrence of stroke. The cytokines, interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), and interleukin-6 (IL-6) are known to have neuromodulatory effects, including the induction of behavioral changes similar to depressive symptomatology. Moreover, increased circulating levels of these cytokines – in particular, IL-6 - have been observed in depressed individuals. Therefore, it is possible that the development of PSD may similarly be associated with increased levels of inflammatory cytokines. In the current study, we recruited 25 AIS patients and assessed executive cognition, clinical depression, and circulating IL-1β, TNFα and IL-6. Each of these variables was measured at three time points following admission for AIS: (i) 1-2 days, (ii) 5-7 days, and (iii) 90 days. Depression was assessed throughout using the Hamilton Depression Scale and Beck Depression Inventory, as well as a structured diagnostic interview for depression (SCID) on Day 90. Cognitive function, including executive function, was measured using the RBANS, Stroop, and Trail Making Tasks. Additional repeated measures of functional and neurological status were obtained using the modified Rankin Scale (mRS) and the National Institute of Health Stroke Scale (NIHSS). Only non-aphasic patients who scored mild-to-moderate on the NIHSS were included. Of those patients that completed all three time points (n=22), seven showed detectable levels of plasma IL-6 within seven days of AIS. A further three patients (13.6%) showed evidence for PSD at Day 90, but none of these had detectable IL-6 at any time point. Contrary to expectations, no patients, at any time point, had detectable plasma

levels of TNFα or IL-1β. Based on evidence that IL-6 may be neuroprotective in animal studies of stroke, the current findings, although based on a small cohort of patients, lend themselves to the novel hypothesis that failure to generate plasma IL-6 elevations after AIS is associated with PSD. Analysis of cognitive and neurological functioning data is ongoing to determine if this relationship holds for other parameters.

Disclosures: S. Norton: None. **R. Glass:** None. **S. Mann:** None. **M. Moccio:** None. **A. Zimmerman:** None. **N. Fiedler:** None. **R. Contrada:** None. **M. Menza:** None. **H. Leventhal:** None. **J. McKinney:** None. **A.W. Kusnecov:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.11/HH14

Topic: C.08.Stroke

Title: A new model to determine the specific and synergetic effects of comorbidities on stroke treatment and outcome

Authors: *T. I. NATHANIEL[1,2], T. COCHRAN[2], J. CHAVES[2], E. FULMER[2], C. SOSA[2], M. FREDWALL[2];
[1]Biomed. Sci., Univ. of South Carolina, Greenville, SC; [2]Univ. of South Carolina Sch. of Med., Greenville, SC

Abstract: Background. Recombinant tissue plasminogen activator (rt-PA) is an effective treatment for patients with acute ischemic stroke. However, the proportion of patients with a stroke treated with rt-PA is incredibly low. This is partly attributed to the role of comorbid conditions as the cause or the consequence of acute ischemic stroke. The specific clinical effect and relationship between comorbidities in stroke, rt-PA treatment and outcome is not known. We investigate this issue in the current study.
Method. First, we characterized comorbidities in an acute ischemic stroke population from a stroke registry, and identified non-cerebrovascular risk factors (comorbidities) that differentiate rt-PA receiving and non-receiving patients. Next, we used matlab programing to develop a new model to determine specific clinical relationships between comorbidities, rt-PA treatment and outcome.
Result. Our model identified how individual and synergetic effects of specific comorbidities (carotid stenosis, congestive heart failure, history of stroke or severity among others) significantly ($P<0.001$) affect rt-PA use and treatment outcome in an individual patient and the stroke population.

Conclusion. Treatment of patients with acute ischemic stroke with rt-PA is dependent on several exclusion criteria. For the first time, we developed a model that can be used to establish the specific and synergetic clinical effects of comorbidities in the use of rt-PA and outcome. This model will play a key role in helping clinicians to make clinical decision in the use of rt-PA and expand its benefits.

Disclosures: T.I. Nathaniel: None. **T. Cochran:** None. **J. Chaves:** None. **E. Fulmer:** None. **C. Sosa:** None. **M. Fredwall:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.12/HH15

Topic: C.08.Stroke

Support: National Institute of Neurological Disorders and Stroke RO1 NS083078

ational Institute of Neurological Disorders and Stroke 41NS064708 (J.C)

National Institute on Aging RO1 AG037506 (M.C.)

American Heart Association grant 14GRNT20460026 (J.C.)

Title: Deficiency of mir-126 exacerbates white matter damage and blood-brain barrier dysfunction after stroke

Authors: *P. YU, C. CUI, M. CHOPP, A. ZACHAREK, P. VENKAT, J. CHEN;
Henry Ford Hosp., Detroit, MI

Abstract: MicroRNAs (miRs) are small non-coding RNA molecules, which regulate gene expression and translation. MiR-126 is primarily expressed in endothelial cells (ECs) and plays a crucial role in regulating the function of ECs, angiogenesis and vascular integrity. In central nervous system (CNS), ATP binding cassette subfamily A member 1 (ABCA1) mediates cholesterol transport, and promotes vascular and axonal/white matter (WM) remodeling after stroke. We hypothesize that inhibition of miR-126 decreases ABCA1 expression, and increases neurological functional deficit, neurovascular and WM damage after stroke. Adult specific conditional EC miR-126 knockout (miR-126$^{EC-/-}$) mice and floxed non miR-126 knockout control (miR-126$^{fl/fl}$) mice were subjected to permanent distal middle cerebral artery occlusion (dMCAo) and were euthanized 4 days after dMCAo. A battery of neurological and cognitive functional tests was measured. To test if miR-126$^{EC-/-}$ regulates vascular and WM changes,

Bielschowsky silver (an axon marker) and Luxol fast blue (a myelin marker), tight junction protein ZO-1, vascular endothelial growth factor (VEGF) and albumin staining were performed. Western blot and PCR were employed to measure miR-126 targets expression in the ischemic brain. Primary cortical neuron (PCN)-axonal outgrowth effects of miR-126 were measured in vitro. miR-126$^{EC-/-}$ mice exhibit significantly increased neurological and cognitive functional deficits after stroke measured by foot-fault, modified neurological severity score and cognitive and odor tests compared to miR-126$^{fl/fl}$ stroke mice. MiR-126$^{EC-/-}$ mice exhibit decreased brain miR-126 and ABCA1 gene expression and significantly increased miR-126 targets, e.g. phosphoinositol-3 kinase regulatory subunit 2, sprouty-related, EVH1 domain containing 1, Monocyte Chemotactic Protein-1 and VEGF protein levels in the ischemic brain compared to miR-126$^{fl/fl}$ stroke mice. MiR-126$^{EC-/-}$ mice also showed significantly decreased axon, myelin density and ZO-1 expression in the ischemic boundary zone and increased BBB leakage compared to WT miR-126$^{fl/fl}$ control stroke mice. In vitro, PCN coculture with mouse brain endothelial cells (BECs) significantly increases axonal outgrowth. However, knockdown of miR-126 in BEC significantly decreases axonal outgrowth compared to coculture with BEC group. Deficiency of endothelial cell miR-126 increases BBB leakage and WM/axonal damage which may contribute to functional deficits after stroke. Concomitant reduction of ABCA1 and upregulation of miR-126 targets may contribute to miR-126 deficiency-induced BBB and WM/axonal damage in ischemic brain.

Disclosures: P. Yu: None. **C. Cui:** None. **M. Chopp:** None. **A. Zacharek:** None. **P. Venkat:** None. **J. Chen:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.13/HH16

Topic: C.08.Stroke

Title: Late stage transient global ischemia causes secondary cell death, characterized by a surge in inflammatory and apoptotic cells, to cerebellum and heart of rhesus macaque monkeys

Authors: *S. MASHKOURI, S. ACOSTA, D. NWOKOYE, Y. KANEKO, C. BORLONGAN; Dept. of Neurosurg. and Brain Repair, Univ. of South Florida, Tampa, FL

Abstract: Stroke is the number one cause of disability and co-morbidities in the adult population and the fourth leading cause of death in the United States. The major pathological consequences of stroke are neurological effects proximal to the infarction area, however, distal areas from the original injury may present pathological manifestations that further increase the risks of co-

morbidities which complicate the patient's health. Transient global ischemia (TGI) model was induced by clipping the arteries originating from the aortic arch. Previously we demonstrated that our TGI model in adult Rhesus macaques (Macaca mulatta) results in marked neuronal cell loss in the hippocampal region, specifically the cornu Ammonis (CA1) region. In the present in vivo study, we evaluated the vulnerability of the cerebellum and the heart for the potential propagation of secondary injuries in late stage TGI model in rhesus macaque monkeys. After 6 months post-injury, the brain and the hearts were harvested and analyzed using immunohistochemical techniques to target infiltrated lymphocytes, apoptosis, and cell death of both cerebellar neurons and cardiac myocyte cells. Unbiased stereological quantification showed significant purkinje cells loss in lobule III and lobule IX of the cerebellum relative to control cerebellum (*p's<0.05). Analysis of gliosis, TNF-alpha, and tunnel revealed significant increase expression in the white matter of lobule III and IX respectively compared to control cerebellum (*p's<0.05). Analysis of the heart revealed significant elevation of activated infiltrated lymphocytes, TNF-alpha, and apoptosis relative to control heart (*p's<0.05), which is indicative of cardiac myocyte damage. The marked increase in inflammatory and apoptotic markers in the cerebellum and the heart suggests a pathological link between the detrimental effects of distal secondary injuries and the development of chronic neuropathological manifestation and cardiac dysfunction. These results further extend our understanding on the propagation of chronic secondary injuries after TGI, highlighting the need to develop therapeutic interventions targeting the brain, as well as the heart, in an attempt to fully address both stroke and its related co-morbidities.

Disclosures: **S. Mashkouri:** None. **S. Acosta:** None. **D. Nwokoye:** None. **Y. Kaneko:** None. **C. Borlongan:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.14/HH17

Topic: C.08.Stroke

Support: NIH RO1 NS083078

NIH RO1NS088656

NIH 41NS064708

American Heart Association grant 14GRNT20460026

Title: Brain and heart interaction after cerebral ischemic stroke-miR-126 effects

Authors: *J. CHEN, C. CUI, X. YANG, A. ZACHAREK, J. XU, P. YU, P. VENKAT, M. CHOPP;
Henry Ford Hosp., Detroit, MI

Abstract: Background: The incidence of cardiovascular diseases are approximately three times higher in patients with neurological diseases than in patients without neurological diseases. Neurogenic stress cardiomyopathy is a well-known syndrome complicating the early phase after an acute brain injury, potentially affecting clinical outcomes. Brain injury can induce cardiac dysfunction. In this study, we investigated mechanisms underlying the effect of the brain on the heart. MiR-126 is emerging as an important factor in the pathogenesis of cardiovascular diseases and stroke. We hypothesize that the decrease of miR-126 after ischemic stroke may mediate "brain and heart interaction", and thereby contributes to cardiac dysfunction after ischemic stroke. Methods: Wild type and miR-126-/- mice were subjected to distal middle cerebral artery occlusion (dMCAo) (n=10/group). Cardiac hemodynamics and function were measured by transthoracic Doppler echocardiography. Mice were sacrificed at 28 days after dMCAo. Immunostaining in heart tissue and microRNA measurements were performed. Results: Mice subjected to stroke exhibited significantly decreased cardiac ejection fraction and increased myocyte hypertrophy and fibrosis as well as decreased cardiac capillary density compared with normal non-stroke mice ($p<0.05$). Stroked mice also exhibited significantly increased heart inflammation and oxidative stress, measured by infiltrating macrophages, 4-hydroxy-2-nonena, NADPH oxidase-2, vascular cell adhesion molecule-1 (VCAM-1), and transforming growth factor β expression compared to non-stroke animals ($p<0.05$). To elucidate the mechanisms underlying cardiac abnormality after stroke, miRNA expression and their target genes were measured. Stroke significantly decreased serum-exosome and heart tissue miR-126 expression, and thereby increased the miR-126 target gene Spred-1, VCAM-1 and monocyte chemotactic protein-1 gene and protein expression in heart tissue compared to non-stroke mice. In addition, specific conditional-knockout endothelial cell miR-126 (miR-126EC-/-) stroke mice exhibited significantly exacerbated heart dysfunction, identified by decreased ejection fraction and shortening fraction, and increased cardiomyocyte hypertrophy and cardiac fibrosis compared to non-miR-126 knockout control (miR-126fl/fl) mice ($p<0.05$). Conclusion: Stroke decreases miR-126 and increases their target gene expression as well as induces cardiac dysfunction. Decreasing miR-126 after brain ischemic stroke may mediate adverse "brain and heart interaction", and thereby contributes to cardiac dysfunction post stroke.

Disclosures: J. Chen: None. **C. Cui:** None. **X. Yang:** None. **A. Zacharek:** None. **J. Xu:** None. **P. Yu:** None. **P. Venkat:** None. **M. Chopp:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.15/II1

Topic: C.09. Brain Injury and Trauma

Support: FP7-HEALTH project 602102 (EPITARGET)

Polish Ministry of Science and Education grant W19/7.PR/2014

Title: Circulating microRNA as a biomarker of epileptogenesis and epilepsy in the rat model of temporal lobe epilepsy

Authors: *K. SZYDLOWSKA, K. NIZINSKA, A. BOT, K. LUKASIUK;
Nencki Inst. of Exptl. Biol., Warsaw, Poland

Abstract: Epilepsy frequently develops as a result of brain insult, ex. brain injury, stroke, inflammation or status epilepticus, however currently there are no tools allowing us to predict which patients suffering from trauma will eventually develop epilepsy or how severe it is going to be. In recent years small non-coding RNAs are proposed as biomarkers for neurological diseases. Particularly microRNAs are interesting candidates, as several of them were described as changing its levels in the brain of epileptic patients and in epilepsy animal models. There is evidence suggesting that microRNAs levels are altered also in the plasma of epileptic subjects, making them attractive candidates for peripheral biomarkers of epilepsy. This study was conducted to evaluate usefulness of plasma miRNAs as biomarkers of epileptogenesis and epilepsy. In our studies we used the rat model of temporal lobe epilepsy. The status epilepticus was evoked by 25 min stimulation of the left lateral nucleus of amygdala (100-ms train of 1-ms biphasic square-wave pulses; 400 μA peak to peak, delivered at 60 Hz every 0.5). Animals were continuously video and EEG monitored for 6 months to detect spontaneous seizures. Blood was collected at 14, 30, 60, and 90 days after stimulation from tail vain. Blood plasma was separated and processed using Affymetrix miRNA 4.1 array strip microarrays. We have compared miRNA levels between sham operated (n=12) and stimulated animals (n=15); $p<0.01$ was used as a cut off. We have detected 14 miRNA differentiating between sham operated and stimulated animals at 14 days, 6 at 30 d, 16 at 60d, and 11 at 90 days. We have also compared the miRNAs levels between animals with high (30-70 seizures/day) and low (1-5 seizures/day) number of seizures. We have found differences in levels of 11 miRNA at 14 d, 7 at 30 d, 11 at 60 d and 8 at 90 d (at $p<0.01$). Levels of miRNA in plasma are altered following epileptogenic stimulus and differentiate between animals with frequent and rare seizures. miRNA may become a useful peripheral biomarker of epileptogenesis/epilepsy as well as severity of the disease. This work was supported by the FP7-HEALTH project 602102 (EPITARGET) and Polish Ministry of Science and Education grant W19/7.PR/2014.

Disclosures: **K. Szydlowska:** None. **K. Nizinska:** None. **A. Bot:** None. **K. Lukasiuk:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.16/II2

Topic: C.08.Stroke

Title: Subarachnoid hemorrhage triggers acute apnea in mice

Authors: ***E. V. GOLANOV**, G. BRITZ;
Neurosurg., Houston Methodist Hosp., Houston, TX

Abstract: Rupture of cerebral aneurysm leading to acute accumulation of blood within the subarachnoid space is known as subarachnoid hemorrhage (SAH). About 44% of SAH victims die within the first few hours following the rupture. Despite the significant progress in repairing ruptured aneurysm, the mortality following SAH remains high. While numerous studies addressed cellular and molecular damages following SAH, relatively little is known of the early events following the aneurysm rupture. We hypothesized that early events following SAH could play a crucial role in the post-ictus mortality. We explored changes in AP, heart (HR) and respiratory rate (RR), regional cerebral blood flow (rCBF) and intracranial pressure (ICP) immediately following the SAH. In spontaneously breathing C57BL adult male mice under isoflurane anesthesia, SAH was triggered by perforation of Willis circle with monofilament. After one hour of observation animals were euthanized, brains harvested, and hemorrhage was scored. Immediately following the perforation ipsilateral rCBF dropped by 75±15%, AP increased by 8±2 %, ICP increased by 102±12 %, cerebral perfusion pressure decreased by 42±7%, and HR increased by 6±3%. Immediately following the perforation, 95% of all animals (117 out of123) demonstrated dramatic decrease in RR by 69±12%, which progressed to apnea in 73% of cases. In animals, which experienced complete respiratory arrest for over 2 minutes, mechanical chest stimulation was required for “resuscitation”. Once the spontaneous respiration was reinstated, animals regained regular RR. This simple maneuver decreased mortality from 46% to 7%. No correlation between the SAH score and RR suppression was observed. No changes in RR or any other parameters were observed in animals with “sham” perforation. The lack of correlation between the size of hemorrhage and very short latency to apnea suggests the neurogenic origin of the phenomenon. Dura mater is heavily innervated by the nerve fibers endowed with TRPV1 ion channels. One hour before the perforation, capsazepine (Czp), blocker of TRPV1 channels (2 μg in 2 μl of aCSF) was injected intraventricularly. Czp failed to modify baseline parameters and acute autonomic responses to SAH. Data reveal severe respiratory

abnormalities in response to SAH. Very short latency of the apneic response and its independence from the size of the hemorrhage strongly suggest neurogenic origin of apnea. Failure of Czp to modify respiratory and other autonomic responses to SAH evidences against involvement of TRPV1 channels. Our data suggest that acute respiratory dysfunction following the ictus could be causative of the high mortality following SAH.

Disclosures: E.V. Golanov: None. **G. Britz:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.17/II3

Topic: C.09. Brain Injury and Trauma

Support: NSF-DMS 1517176

Title: Laminar profile underlying the propagation of CSD

Authors: *D. RAMOS[1], S. GARCIA[1], Y. FROMETA[2], J. HOW[3], J. RIERA[1], Y. MORI[4]; [1]Biomed. Engin., [2]Florida Intl. Univ., Miami, FL; [3]UCSD, San Diego, CA; [4]Univ. of Minnesota, Minneapolis, MN

Abstract: Cortical Spreading Depression (CSD) is a wave of complete neuronal depolarization that usually lasts for one to two minutes and can silence brain activity for a certain time after its occurrence. Sixty years after the initial discovery by Antonio Leão, the mechanisms for CSD propagation are still elusive. In this research project, we perform multisite recording of electric potentials to study laminar features of relevance for the CSD propagation in the cerebral cortex of rats. We perform two craniotomies on the same brain hemisphere. We drop 20-40 µL of potassium acetate (1M) on one of the craniotomies, which induces a CSD. Using an acute silicon-based electrophysiological probe, we record electric potentials from the other craniotomy while the CSD propagates through it. We perform spike sorting to determine the silencing patent of single neurons. Current source density analysis allows us to investigate disruptions in the spatial profiles of postsynaptic potentials. We perform laminar comparison of spiking rates and postsynaptic activities before, during and after a CSD. We have consistently obtained in our results a laminar propagation of CSD as can be seen in Figure 1, which shows a negative current from the supra granular layer to the infra granular layer during the CSD event. In addition, we have observed different spiking rates during the different phases of CSD as its represented in Figure 2. It is believed that with a better understanding of the mechanisms for CSD propagation is critical to create effective therapeutic strategies for related brain disorder such as epilepsy,

stroke and migraine. Our data will be useful in the future to calibrate a computational model, which is currently being developed at the University of Minnesota.

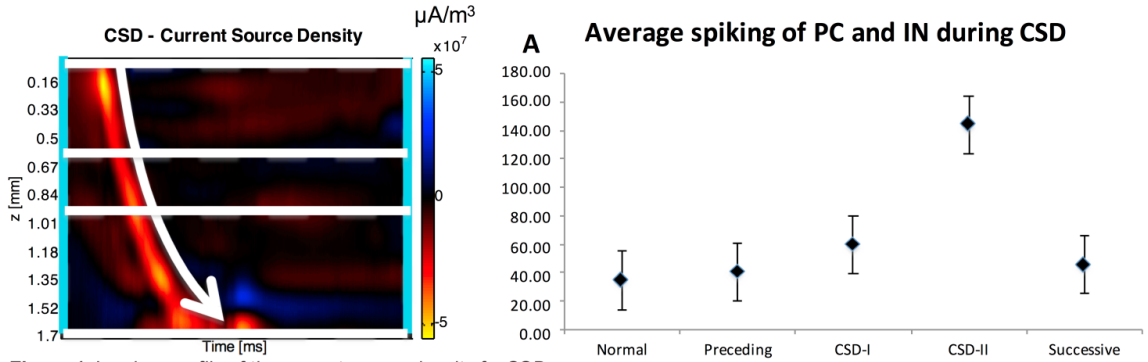

Figure 1. *Laminar profile of the current source density for CSD.*

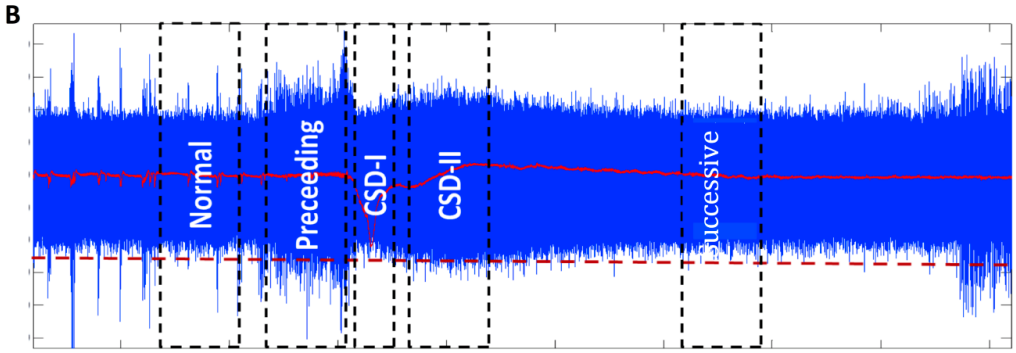

Figure 2. A. *Spiking rate (spike/sec) of Pyramidal cells (PC) and Interneurons (IN) during the different phases of CSD as demonstrated in* ***Figure 2.B.***

Disclosures: D. Ramos: None. **S. Garcia:** None. **Y. Frometa:** None. **J. How:** None. **J. Riera:** None. **Y. Mori:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.18/II4

Topic: C.08.Stroke

Title: Changes in the extracellular matrix following ischemic stroke in the rat

Authors: *E. M. ANDREWS, A. MEIER, T. BRUGMAN, M. PATEL;
Midwestern Univ., Downers Grove, IL

Abstract: Ischemic strokes results in immediate inflammation and neuronal cell death. The amount of functional recovery that is seen following a central nervous system (CNS) injury is limited. Recent studies have shown that chondroitin sulfate proteoglycans (CSPGs) contribute to the lack of functional plasticity due to their inhibition of neurite outgrowth. Previous research indicates that there is limited neuroplasticity (neurite outgrowth) from the uninjured contralateral hemisphere and this neuroplasticity can be enhanced with various therapeutic treatments (anti-nogo, adult human bone marrow stem cells, etc.). Astrocytes and microglia are involved in the post-ischemic inflammatory response, and contribute to the changing extracellular matrix (ECM) by both producing CSPGs and producing enzymes that can alter CSPGs and the ECM in the ischemic core and penumbra region. Alterations to CSPG expression, the ECM and the glial response may be altered in the contralateral sensorimotor cortex to allow for the limited neuroplasticity seen after injury.
In the present study, we investigated glial activation and CSPG expression in the sensorimotor cortex after an ischemic stroke in the rat, both ipsilateral and contralateral to the lesion 3, 7, 14 and 28 days post stroke. Both neurocan expression and GFAP expression increased at 14 days in the ipsilateral sensorimotor cortex. Total neurocan expression is significantly increased 14 days post stroke in the ipsilateral sensorimotor cortex as compared to control. GFAP expression (indicative of activated astrocytes) is significantly increased in the sensorimotor cortex surrounding the lesion at 14 days post stroke as compared to control. Both aggrecan and CD11b (microglia) expression are stable at 3 and 14 days post stroke as compared to controls. Western blot analysis of CSPG expression at 7 days and 28 days post injury is under investigation. CSPG distribution at all time points (3 day, 7 day, 14 day, 28 days post injury) is being analyzed via immunohistochemistry. These results may help pinpoint the best time and location of treatment to help restore function following an ischemic stroke.

Disclosures: E.M. Andrews: None. **A. Meier:** None. **T. Brugman:** None. **M. Patel:** None.

Poster

519. Stroke and Injury Bio-Markers: Electrophysiological and Neurochemical Approaches

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 519.19/II5

Topic: C.09. Brain Injury and Trauma

Support: Veterans Affairs Grant 1I01RX000511

Title: Memantine therapy as a tool to break the link between brain injury and Alzheimer disease: a preclinical study in humanized amyloid-beta mice

Authors: E. E. ABRAHAMSON[1,3], L. SHAO[1], S. CULVER[2], W. PALJUG[1], X. MA[2], C. E. DIXON[2], *M. D. IKONOMOVIC[1,3];
[1]Neurol., [2]Neurosurg., Univ. of Pittsburgh, Pittsburgh, PA; [3]Grecc, VA Pittsburgh HS, Pittsburgh, PA

Abstract: Traumatic brain injury (TBI) results in brain accumulation of amyloid-β (Aβ) peptides and neuronal degeneration which could contribute to the risk of developing Alzheimer's disease (AD) later in life. Memantine is a clinically well-tolerated, moderate-affinity, noncompetitive glutamate receptor antagonist proven beneficial in excitotoxicity models and is one of the few currently available therapies for AD, but its utility in ameliorating Aβ- and glutamate-related excitotoxic neurodegeneration and in improving long term functional outcome after TBI has not been explored. We measured human Aβ concentration and assessed histological and behavioral outcomes after controlled cortical impact (CCI) injury in human Aβ (hAβ) knock-in mice treated with memantine hydrochloride (memantine) or vehicle (saline). Adult hAβ mice received memantine (2.5, 5, 10 mg/kg) or vehicle by intraperitoneal injection daily for 3 weeks starting 1 hr after CCI injury (depth = 1.6 mm; velocity = 6.0 m/s). Relative to naïve mice, brain concentrations of Aβ1-42 and Aβ1-40 were elevated in CCI/vehicle group. The 5 mg/kg dose of memantine significantly lowered Aβ1-42 concentration in cerebral cortex ipsilateral to injury ($p<0.01$) and reduced cortical lesion size compared to the CCI/vehicle group. CCI-induced reductions in hippocampal synaptic densities (regions CA1 and CA3) were moderately attenuated with the 2.5 and 5 mg/kg doses. CCI-induced impairment of vestibulomotor function was not improved by memantine on days 1-5 after injury, while all doses of memantine moderately attenuated deficits in novel object recognition test performance on days 9 and 10 after injury. Memantine administered at a dose of 5 mg/kg significantly ameliorated CCI-induced spatial memory impairments on the Morris water maze acquisition and probe tests (both tests $p<0.05$) on days 14-20 after injury. Collectively, these results demonstrate neuroprotective and behavioral recovery-enhancing effects of daily treatment with 5 mg/kg memantine over three weeks after severe TBI. These results provide preclinical support for use of memantine to improve outcome after TBI and to reduce the risk for AD.

Disclosures: E.E. Abrahamson: None. **L. Shao:** None. **S. Culver:** None. **W. Paljug:** None. **X. Ma:** None. **C.E. Dixon:** None. **M.D. Ikonomovic:** None.

Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 520.01/II6

Topic: C.08.Stroke

Support: VA CS R&D I01 CX000586-01A1

Title: Analyzing chronic stroke white matter lesions using a HARDI streamline database

Authors: *T. J. HERRON[1], N. DRONKERS[3,4,5], A. U. TURKEN[2];
[1]Neurology, Res., [2]Res. & Develop., VA Northern California Hlth. Care Syst., Martinez, CA; [3]Ctr. for Aphasia & Related Disorders, VA Northern California, Martinez, CA; [4]Neurol., UC Davis, Davis, CA; [5]Neurolinguistics, Natl. Res. Univ. Higher Sch. of Econ., Moscow, Russian Federation

Abstract: We built a HARDI diffusion streamline database from 43 healthy controls for identifying likely connectivity deficits of chronic stroke patients who have lesion location masks available that specify brain damage. We verified the accuracy of the lesion mask estimated connectivity deficits in 40 stroke patients by comparison to the patients' HARDI deficits vs. age and sex matched controls. We then applied the streamline database to study a separate group of 191 left hemisphere chronic stroke subjects who suffered dysphasia and were imaged to generate hand-delineated lesion masks. We found that estimated white matter (WM) connectivity deficits contribute to explaining, along with regional gray matter (GM) damage and lesion size, behavioral speech and language deficits in the patient group.
HARDI images were processed using mrtrix to obtain 500,000 streamlines/subject, seeded at random WM and GM locations. Streamlines were partitioned in native space using the Harvard-Oxford atlas specifying 48 cortical GM locations and 7 subcortical GM locations per hemisphere. After further filtering and transformation, 6.5 million streamlines traversing 190,925 possible MNI locations were produced and classified according to which pairs of the 110 GM locations they connect. The Table shows the results of verifying the database in our 40 stroke cohort: lesion mask estimated connectivity deficits correlate well with those found directly from patient HARDI data.
When applied to the 191 patient chronic stroke cohort with lesion masks and Western Aphasia Battery (WAB) scores, we found that WM lesion size is more important than GM lesion size in predicting speech and language deficits, and that GM ROI disconnectivity is more critical than GM ROI damage, as illustrated in the Figure.

LH lesion size	LH GM %	Corr_LH	Corr_LH-RH	Corr_RH	RH lesion Size	RH GM %
379.6	64.1	0.66	0.36	-0.10	1.0	95.0
268.3	58.5	0.77	0.48	0.19	2.2	9.1
239.0	46.0	0.66	0.42	0.09	0.0	NaN
231.3	55.8	0.53	0.39	0.08	0.0	NaN
202.4	55.4	0.35	0.33	0.00	0.1	0.0
138.6	59.6	0.66	0.27	0.03	0.0	NaN
136.4	48.7	0.45	0.09	-0.01	0.0	NaN
130.4	49.2	0.72	0.39	0.01	0.0	NaN
111.8	43.4	0.74	0.43	-0.05	0.0	0.0
110.8	64.5	0.54	0.28	0.01	0.0	NaN
108.5	46.4	0.61	0.27	0.21	0.0	NaN
96.2	40.8	0.50	0.27	-0.05	0.0	NaN
95.6	68.0	0.31	0.11	-0.06	0.0	NaN
95.6	68.0	0.40	0.19	-0.05	0.0	NaN
94.9	54.1	0.49	0.25	-0.05	0.0	NaN
86.4	43.1	0.51	0.41	0.07	8.6	6.2
70.7	47.0	0.40	0.06	0.10	0.1	0.0
69.6	66.4	0.39	0.15	-0.04	0.0	NaN
69.2	32.0	0.53	0.30	-0.06	0.5	0.0
60.8	38.0	0.58	0.41	0.08	0.0	NaN
57.8	65.4	0.43	0.07	-0.12	0.0	NaN
51.5	40.6	0.21	0.21	0.10	2.9	0.0
44.7	65.5	0.51	0.27	0.09	0.0	NaN
37.1	61.2	0.25	-0.15	0.00	0.0	NaN
29.7	46.9	0.45	0.24	0.02	0.0	NaN
24.9	2.4	0.00	-0.10	0.30	104.6	37.1
20.8	2.8	0.40	0.40	0.05	0.0	NaN
19.1	60.2	0.47	0.05	-0.10	0.0	NaN
15.0	62.2	0.11	0.03	-0.08	1.0	6.2
13.1	25.9	0.42	0.19	0.07	0.1	42.9
11.4	63.3	0.00	0.09	-0.21	0.3	4.8
9.9	80.2	0.19	0.01	-0.12	0.2	14.3
5.1	65.2	0.15	0.07	0.06	0.0	NaN
4.0	56.2	0.08	0.07	-0.02	0.0	NaN
1.0	49.2	0.14	0.08	0.05	0.0	NaN
0.3	0.0	-0.01	0.07	0.25	78.1	64.9
0.2	0.0	-0.06	-0.10	-0.05	2.5	95.8
0.1	46.2	0.19	0.13	0.09	0.3	81.1
0.0	NaN	-0.02	0.02	0.19	16.2	67.9
0.0	NaN	-0.13	0.34	0.51	244.5	51.1

Table: Table of 40 Patient Lesion sizes per hemisphere (columns 1 and 6, in cm^3) along with approximate lesion % being GM (columns 2 and 7) with correlations (Spearman) between streamline deficits (direct DTI measured vs lesion mask + database estimated) both within hemisphere (columns 3 and 5) and between hemispheres (column 4), with the correlations being across 48 H-O cortical and 7 subcortical parcellations.

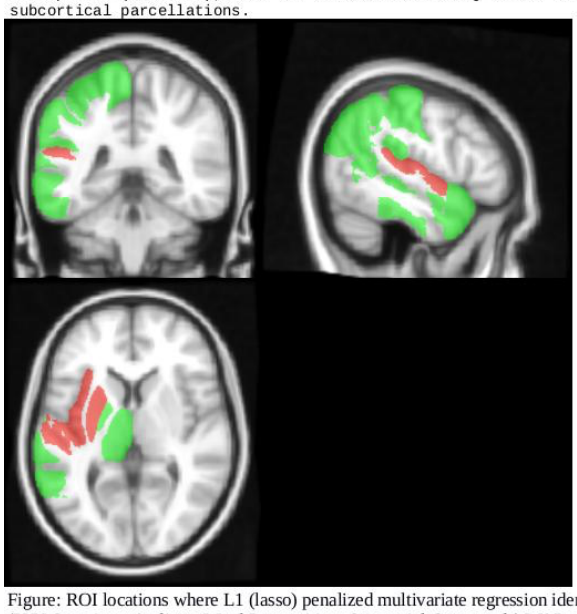

Figure: ROI locations where L1 (lasso) penalized multivariate regression identifies Comprehension (Y/N Questions, Auditory Word Recognition, Sequential Commands) WAB subtest deficits. Blue =GM damage affects Comprehension; Green = GM Disconnection (i.e. WM damage) Comprehension; Red = Both Damage and Disconnection affects Comprehension. WM lesion size and then GM lesion size were the two most important predictors, followed by the above ROIs.

Disclosures: T.J. Herron: None. **N. Dronkers:** None. **A.U. Turken:** None.

Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 520.02/II7

Topic: C.08.Stroke

Support: American Heart Association Grant 15GRNT25700431

Title: Free-water and free-water corrected fractional anisotropy within the primary and secondary corticospinal tracts predict grip strength in chronic stroke.

Authors: *D. B. ARCHER, S. A. COOMBES;
Univ. of Florida, Gainesville, FL

Abstract: It is well documented that a stroke causes changes in brain structure, and that motor deficits are related to the amount of damage found within the corticospinal tracts (CSTs). Conventional diffusion MRI stroke studies often calculate measures of fractional anisotropy and diffusivity within specific regions of the CST such as the PLIC and CP. Lower FA and higher diffusivity in these regions have been associated with poorer motor function. In the current study we extend this literature by addressing four unresolved issues. 1) The single tensor model does not control for free-water, so it is not clear from these studies whether the deficits in function relate to changes in tissue and/or fluid. 2) It is not clear whether correlations between microstructure and function are limited to the PLIC and CP or extend to other regions of the CST. 3) PLIC and CP regions have been drawn across primary and secondary CSTs, and so it is not clear if microstructure in both tracts equally relate to function. 4) There is currently no consensus on whether lesions should be masked or not prior to calculating and then correlating microstructure with function. To address these issues, we calculated free-water and free-water corrected FA within every axial slice of the M1-CST and PMd-CST. We then correlated free-water and free-water corrected FA within every slice for both tracts with grip strength in twenty-three individuals post-stroke. We found that free-water and free-water corrected FA from five segments of the M1-CST and two segments of the PMd-CST predicted post-stroke grip strength ($p<0.05$, corrected). These segments were located within the CP, PLIC, and corona radiata. All segments were inputted as independent variables in a multiple regression analysis with bidirectional elimination to predict grip strength, in which we found that the optimal model ($R_{adj}^2=54.85\%$, $p<0.01$) included free-water in the M1-CST PLIC, free-water corrected FA in the the M1-CST CP, and free-water in the PMd-CST cortex. Our observations show that a data-driven approach that separately assesses tissue and free-water is robust to outliers and provides a more detailed understanding of the relationship between structure and function in chronic stroke.

Disclosures: D.B. Archer: None. **S.A. Coombes:** None.

Poster

520. Stroke Imaging and Diagnostic Studies

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Program#/Poster#: 520.03/II8

Topic: C.08.Stroke

Support: National Scientist Development, from American Heart Association, #12SDG12080169

Research fellowship from the Uehara Memorial Foundation

Title: Changes in resting-state fMRI in acute subcortical stroke correlate with long term motor recovery

Authors: *K. AKAZAWA[1], J. XU[2], M. BRANSCHEIDT[3,4], T. KITAGO[5], P. CELNIK[3], A. LUFT[4], J. KRAKAUER[2], A. V. FARIA[1];
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Abstract: Resting-state functional MRI could potentially provide information on the mechanisms of motor acute deficits and recovery after acute stroke. Acute changes in connectivity between ipsilesional motor area and other cortical regions, and correlations between connectivity status and motor outcomes have been reported. One obstacle of studying resting fMRI in stroke is that the cortical lesion itself may be a confounder. Here, we explored the acute changes in resting fMRI and neural correlates of motor recovery in participants with subcortical strokes.

Resting fMRI, and Fugl-Meyer (FM) scores were obtained from 10 patients with supra tentorial subcortical strokes (mean age: 61.7 ± 5.44, 5 males) within 1, 4, 12, 24, and 52 weeks after stroke. Eleven age-matched healthy controls (62.7 ± 8.04, 7 males) were scanned at the same intervals. The brain segmentation and structure-based resting fMRI analysis were performed automatically in BrainGPS (www.MRICloud.org).

We first evaluated differences between patients and controls at first week, regarding resting fMRI connectivity (z-transformed Pearson correlations) between the ipsilesional precentral gyrus and 27 gyri in the ipsi- and contra-lateral frontal and parietal lobes. We then correlated the significant different connections (p-values<0.05 after Bonferroni correction) from resting fMRI with acute FM scores, and with motor recovery as measured by (FM at 24 weeks - acute FM)/acute FM. Finally, we tested the value of these connections on improving a predictive model of motor function based on acute FM scores only.

Correlations between ipsilesional precentral and ipsi and contralesional postcentral gyrus, and those between ipsilesional precentral and supramarginal gyrus were significantly different between patients and controls (p=0.014, 0.031, 0.048, respectively). This indicates that although the cortex was not directly affected, there were acute changes in connectivity between primary

motor cortex and primary sensory cortex intra and inter-hemisphere.
The acute status of connectivity between ipsilesional precentral and postcentral gyrus did not correlated with acute FM score, but it did correlated with long term (~6 months) motor recovery (r=0.825, p=0.035). As expected, the acute FM scores predicted FM scores at 6 moths, and additional resting fMRI information did not improve the model. Our results suggest that acute cortical motor-sensory connectivity may be involved in functional motor recovery, although additional connectivity measurements seem to be redundant to predict motor outcomes.

Disclosures: **K. Akazawa:** A. Employment/Salary (full or part-time): Johns Hopkins University, Doctor-NET Inc.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; The Uehara Memorial Foundation. **J. Xu:** A. Employment/Salary (full or part-time): Johns Hopkins University. **M. Branscheidt:** A. Employment/Salary (full or part-time): Johns Hopkins University, University Hospital Zurich. **T. Kitago:** A. Employment/Salary (full or part-time): Columbia University Medical Center. **P. Celnik:** A. Employment/Salary (full or part-time): Johns Hopkins University. **A. Luft:** A. Employment/Salary (full or part-time): University Hospital ZurichZurich. **J. Krakauer:** A. Employment/Salary (full or part-time): Johns Hopkins University. **A.V. Faria:** A. Employment/Salary (full or part-time): Johns Hopkins University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; American Heart Association.

Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 520.04/II9

Topic: C.08.Stroke

Title: Study of cerebral blood flow patterns in patients with transient neurological dysfunction assessed by arterial spin labeling perfusion MRI

Authors: *K. SUMIYOSHI;
Natl. Disaster Med. Ctr., Tachikawa, Japan

Abstract: Purpose: It can be difficult to differentiate epilepsy from other diseases that cause transient neurological dysfunction. An interictal electroencephalogram does not always provide evidence for diagnosis of epilepsy. It has been reported that perfusion changes could be detected for a time after periictal period of epilepsy using arterial spin labeling (ASL) perfusion MRI. We

aimed to examine cerebral blood flow patterns in patients with transient neurological dysfunction. **Methods:** One-hundred cases with transient neurological dysfunction were studied. Patients were imaged at a 3T MRI including ASL, and were also examined using EEG. The abnormal findings of ASL were compared with those obtained from EEG. **Results:** We found temporal changes of perfusion pattern in patients of epilepsy. On the other hand, there were almost no changes in patients of syncope. **Conclusion:** The combined use of EEG and ASL can aid the differential diagnosis of epilepsy.

Disclosures: K. Sumiyoshi: None.

Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 520.05/II10

Topic: C.08.Stroke

Title: ENIGMA Stroke Recovery: Big data neuroimaging to predict stroke recovery

Authors: *S.-L. LIEW[1], N. JAHANSHAD[1], J. ANGLIN[1], N. KHOSHAB[2], B. KIM[1], W. NAKAMURA[1], H. NHOUNG[1], J. RONDINA[3], C. TRAN[1], M. BORICH[4], L. BOYD[5], S. C. CRAMER[2], M. A. DIMYAN[6], E. ERMER[6], C. E. LANG[7], J. LI[1], T. NICHOLS[8], P. ROBERTS[9], N. SANOSSIAN[1], S. SOEKADAR[10], N. WARD[3], L. T. WESTLYE[11], C. WINSTEIN[1], G. F. WITTENBERG[6], P. M. THOMPSON[1];
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Abstract: A persistent challenge in stroke neurorehabilitation research is the vast heterogeneity of the post-stroke population. Previous studies have suggested inconsistent relationships between post-stroke neuroanatomy and motor recovery based on smaller samples. However, large, diverse datasets can provide the statistical power to robustly form and evaluate these hypotheses. The ENIGMA Stroke Recovery Working Group uses innovative meta-analytic approaches to generate large datasets (goal n>3000) of neuroimaging and behavioral data, collected across multiple study sites (http://enigma.ini.usc.edu/ongoing/enigma-stroke-recovery/). Here, we present preliminary findings relating post-stroke neuroanatomy with upper limb motor impairment. Based on previous work, we hypothesize that regions of the basal ganglia should most strongly relate to motor impairment. Structural T1-weighted MRIs from over 1400 patients

across 12 sites have been committed. Data from 251 stroke patients across 8 research samples were included in this analysis. ENIGMA protocols were used to extract subcortical measures and perform quality control checks. Regression analyses examined subcortical volume as a predictor of motor impairment; additional covariates included age, sex, time since stroke, hemisphere affected, and total intracranial volume. Focal effects of each lesion on the brain volumes were manually marked and included in the model (volume=0); 10,000 permutations were used to obtain a non-parametric estimate of the statistical significance. In line with our hypotheses, we found a number of significant associations between subcortical volume and motor impairment, particularly in the basal ganglia and lateral ventricles. Importantly, individually analyzing three of the largest samples (n=37, n=34, n=27) yielded weak and inconsistent results across samples (Table 1). These preliminary results demonstrate the feasibility and utility of integrating multi-site data to study the relationship between post-stroke neuroanatomy and motor impairment. We further demonstrate the variability of findings across individual samples and the improved ability of this combined approach to more sensitively identify post-stroke brain-behavior relationships.

Table 1. Subcortical regions related to post-stroke motor behavior. Results relating subcortical regions to motor behavior are shown for the combined sample (n=251) as well as three of the largest individual sites. Beta value listed is the observed slope of the regression.

Combined Sample (n=251)

Region	*P-value*	*β-value*
L caudate	0.0293	751.67
L lateral ventricle	0.0175	-5559.06
R nucleus accumbens	0.0427	91.21
R lateral ventricle	0.0282	-5668.08
R pallidum	0.0153	261.03
R putamen	0.0098	1065.59
R thalamus	0.0672	797.97

Research Sample 1 (n=34)

Region	*P-value*	*β-value*
L lateral ventricle	0.058	-13109.71

Research Sample 2 (n=27)

Region	*P-value*	*β-value*
L caudate	0.028	4444.69

Research Sample 3 (n=37)

Region	*P-value*	*β-value*
R pallidum	0.052	639.12

Disclosures: S. Liew: None. **N. Jahanshad:** None. **J. Anglin:** None. **N. Khoshab:** None. **B. Kim:** None. **W. Nakamura:** None. **H. Nhoung:** None. **J. Rondina:** None. **C. Tran:** None. **M. Borich:** None. **L. Boyd:** None. **S.C. Cramer:** None. **M.A. Dimyan:** None. **E. Ermer:** None. **C.E. Lang:** None. **J. Li:** None. **T. Nichols:** None. **P. Roberts:** None. **N. Sanossian:** None. **S. Soekadar:** None. **N. Ward:** None. **L.T. Westlye:** None. **C. Winstein:** None. **G.F. Wittenberg:** None. **P.M. Thompson:** None.

Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 520.06/II11

Topic: C.08.Stroke

Title: Longitudinal monitoring of mesoscopic cortical activity using a fluorescent bead mouse model of small vessel disease and GCaMP6 imaging

Authors: *M. BALBI, G. SILASI, Y. SEKINO, M. VANNI, J. LEDUE, T. H. MURPHY; Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Small vessel diseases (SVDs) comprise the majority of cases of vascular dementia, itself the second most common form of dementia. Obstruction of small vessels leads to cell death from oxygen starvation and contributes to vascular dementia. These microinfarcts usually escape detection by conventional magnetic resonance imaging and are identified mostly upon postmortem examination. Reliable animal models that allow high-resolution functional imaging are needed to study brain function in SVD. Our work explores a SVD model where occlusions of small penetrating arterioles are reproduced in mice by endovascular injection of 20 μm fluorescent microspheres. Occlusions in this model have been shown to cause gross motor impairments as well as cell loss and axonal disruption throughout the brain. While the cortex itself is relatively spared from structural damage —less than 1% of occlusions result in

microinfarcts[1]— subcortical, white matter damage may disrupt cortical function. Hence we evaluated functional connectivity in the mouse cortex using genetically encoded calcium indicators. Mesoscopic functional connectivity was mapped longitudinally in awake GCaMP6 mice using transcranial wide-field calcium imaging through a bilateral chronic window implant. Spontaneous activity was recorded over 4 weeks before and 6 weeks after injection of red fluorescent microspheres (2000, 20 μm microspheres in 100μl PBS) into the left common carotid artery. Microsphere occlusions were quantified with optical coherence tomography (OCT) and changes in cerebral blood flow were assessed with laser speckle imaging. A battery of behavioral tests were performed to detect functional impairments. We assessed motor function using the clasping and neurodeficit scores (NDS). The NDS in stroke mice was significantly higher ($p=0.03$) than in sham mice. Stroke mice showed a trend to a higher clasping score, with no sham mice scoring above 0. We analyzed seed pixel correlation and standard deviation maps within somatosensory, visual, and motor cortices but no significant differences were found between sham and stroke groups. A possible interpretation of our findings is that impairments to functional circuits in subcortical structures such as the basal ganglia or thalamus affect motor function without alterations to functional connectivity in the cortex detectable at the mesoscopic scale. Analysis at the microscopic scale may bring to light more subtle relationships and provide a unifying theory spanning microscopic, mesoscopic and behavioral phenomena. [1] Silasi et al., JCBFM (2015)35,734-738

Disclosures: **M. Balbi:** None. **G. Silasi:** None. **Y. Sekino:** None. **M. Vanni:** None. **J. LeDue:** None. **T.H. Murphy:** None.

Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 520.07/II12

Topic: C.08.Stroke

Support: Ressler Family Foundation

Title: *In vivo* calcium imaging reveals distributed cortical network dynamics after stroke

Authors: ***S. LATIFI**[1], A. M. ESTRADA-SÁNCHEZ[2], E. DONZIS[2], M. S. LEVINE[2], P. GOLSHANI[1], S. T. CARMICHAEL[1];
[1]Neurol., UCLA David Geffen Sch. of Med., Los Angeles, CA; [2]IDDRC, Semel Inst. for Neurosci. and Human Behavior, BRI, UCLA, Los Angeles, CA

Abstract: Ischemic stroke causes cell death, massive disconnections of individual neurons and disrupted functional circuits. Although functional mapping studies at macroscopic and mesoscopic scales demonstrate that plasticity within cortical regions after stroke leads to partial recovery of function after the initial injury, the role of single neurons in response to altered brain networks, or the cortical connectome, is not clear. Here we used *in vivo* two-photon calcium imaging in head-fixed freely moving adult mice to show how the response of individual neurons could affect neural network topology after stroke. Calcium dynamics were evaluated in peri-infarct motor, and more distant premotor and somatosensory cortex before and after inducing stroke in the forelimb motor cortex. These three cortical areas represent distinct circuits in relationship to the stroke (adjacent and distant) and are involved in the axonal plasticity that underlies recovery. While diminished network functional connectivity was observed for all these cortical areas, the response of functional hubs showed different signaling patterns. Based on the amplitude of change in calcium transients ($\Delta F/F$), a decrease was found only in the somatosensory cortex. Peri-infarct motor and premotor cortex exhibited a decrease in frequency whereas an increase was observed in somatosensory cortex. Applying graph theoretical analysis, our data confirmed that before stroke, cortical networks followed a high clustering pattern with heavy-tailed degree distributions. After stroke, cortical networks lose their tightly correlated firing patterns among neurons, with each area showing a distinctive pattern of loss in correlated network firing over time. Finally we evaluated mean correlation coefficients as a function of distance between neurons. Calcium transients were assessed during resting and limb motion epochs before and after stroke. We observed that the average correlation at all distances (100-400 μm) was increased during motion after inducing stroke compared to the resting state. However before stroke the average correlation was higher at resting state than during limb motion . Altogether, our data suggest that stroke disrupts network topology and causes sparse network in the areas adjacent to stroke. Furthermore, our findings are geared towards understanding recovery potential and correlation in network gain over time. Supported by the Ressler Family Foundation.

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Poster

520. Stroke Imaging and Diagnostic Studies

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Program#/Poster#: 520.08/II13

Topic: C.08.Stroke

Support: Ontasian Imaging Lab

Stroke Imaging Lab for Children (SILC)

Rani Ghar Cerebral Palsy Foundation

Title: Hyperventilation cerebrovascular reactivity in children with Moyamoya

Authors: P. SHAH-BASAK[1], N. DLAMINI[2], M. MOHARIR[3], P. DIRKS[4], M. SHROFF[1], G. DEVEBER[2], *W. J. LOGAN[5];
[1]Diagnos. Imaging, [2]Neurol., [4]Neurosurg., [3]The Hosp. for Sick Children, Toronto, ON, Canada; [5]Hosp for Sick Children, Toronto, ON, Canada

Abstract: The objective of this study was to evaluate the utility of hyperventilation (HV) as a stimulus for the determination of cerebrovascular reactivity (CVR) in children with cerebral vasculopathy. Breath hold (BH) has been a useful and effective technique for determining CVR (Thomas et al., 2013). Some children however are unable to perform BH successfully while still able to perform HV. In order to determine whether HV is an effective stimulus for CVR it was compared to BH in a series of 5 patients. These patients had various degrees of Moyamoya, a progressive cerebral vasculopathy.

Seven CVR studies were performed in five children in order to monitor the effect of their vasculopathy on their cerebrovascular reserve capacity. The children ranged in age from 11 to 16 (3 girls) mean age = 13.6 ±2.51 years). Two of the patients had revascularization surgery to improve their cerebral circulation. CVR was determined using an MRI technique which detects the BOLD signal change and reflects the hemodynamic change produced by BH induced CO2 alteration. BH studies involved epochs of 17-25 sec of BH followed by periods of normal breathing for a total of 5 epochs during the BOLD image acquisition. Two BH studies were performed and compared in each CVR study except one. HV studies were performed by instructing the patients to breathe fast and deep for 20 -30 sec followed by normal breathing for a total of 6-9 epochs over 6 minutes. The images were analyzed by comparing the MR signal during the task with that during normal breathing. The images were processed using STIMULATE software (Strupp, 1996). The signal change produced by the task (BH or HV) was expressed as a percent of the MR signal during normal breathing. The parametric images of each study were judged normal or abnormal by visual inspection.

The mean signal changes for the 2 BH studies were 2.2% (1.7-2.8%) and 2.4% (1.5-3.1%), respectively. These means did not differ significantly (Wilcoxon sign rank test; $p>0.05$). The mean signal change for HV was 2.7% (1.7-3.9%) and it did not differ from BH ($p>0.05$). Two CVR BH studies were normal, three were abnormal and two were slightly abnormal. There was a 100% concordance for the six replicate BH studies. The HV studies were concordant with the BH studies except in one patient where the CVR on HV study was slightly abnormal while that based on BH was found to be abnormal.

In conclusion, HV can produce a MR signal change comparable to that produced by BH and appears to be an effective technique for determining CVR in those children who cannot successfully perform BH.

References: 1) Thomas B, Logan W, Donner E, Shroff, M (2013). Childs Nerv Syst. 29(3), 457-63; 2) Strupp JP (1996). NeuroImage, 3.

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Poster

520. Stroke Imaging and Diagnostic Studies

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Program#/Poster#: 520.09/II14

Topic: C.08.Stroke

Support: NIH Grant HD039343

Title: Whole brain and brainstem morphological changes in chronic hemiparetic stroke

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Abstract: Previous studies using functional neuroimaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), have frequently reported increased motor-related neural activity in motor areas of both ipsilesional and contralesional hemispheres in stroke patients compared to healthy subjects. The neuroimaging studies over the past two decades have found this increased brain activity in more impaired chronic stroke patients more evident in contralesional than ipsilesional hemisphere. More recent studies have suggested that this relative shift in cortical activity to contralesional sensorimotor cortex in individuals with stroke is correlated to the shoulder abduction loading level in the paretic upper limb. This alternative brain activity in individuals with chronic stroke over an extended period of time, may lead to brain structural changes. However, which gray matter regions of brain and brainstem are involved in this cortical shift has not been studied yet. The goal of this study therefore was to use Voxel-Based Morphometry (VBM) analysis on anatomical magnetic resonance images to identify the whole brain and brainstem gray matter density changes over both hemispheres. 10 chronic (>1yr) stroke patients and 10 age-matched healthy controls were recruited to participate in this study and their MRI scans were collected on a 3T Siemens Trio magnetic resonance scanner at the Northwestern University Center for Translational Imaging. High resolution T1-weighted anatomical scans were obtained using a multi-shot gradient echo (GE) sequence with TR = 0.8 s, TE = 2 ms, matrix size = 256 x 256 x 176, and voxel size = 1 x 1 x 1 mm. VBM 8 on SPM12 was used to segment structural images to gray matter, white matter and CSF tissues. Segmented images were modulated and normalized to MNI space while preserved for the total amount of tissue. Normalized gray matter images were smoothed with 6 mm FWHM Gaussian kernel and then used in a between-group two sample t-tests to compare the voxel-wise gray matter density changes in stroke patients compared to control group. Using

voxel-wise corrected p value < 0.05 and p cluster-level corrected < 0.05, the brain and brainstem regions with increase/decrease gray matter density were identified. The stroke patients compared to healthy controls showed significant gray matter density changes in sensorimotor cortices, premotor area, thalamus and bulbar nuclei. Subsequent work will test the relationship between changes in neural morphology in the lesioned and non-lesioned hemispheres and shoulder abduction induced reductions in reaching and hand opening and grasping in individuals with chronic stroke.

Disclosures: H. Karbasforoushan: None. **J.P.A. Dewald:** None.

Poster

520. Stroke Imaging and Diagnostic Studies

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Program#/Poster#: 520.10/II15

Topic: C.08.Stroke

Support: MEXT KAKENHI Grant Number 16K19995

Title: Neurite orientation dispersion and density imaging revealed the brain microstructural ischemic damage of moyamoya disease

Authors: *S. HARA[1], T. NARIAI[1], S. MURATA[2], K. TSURUTA[2], Y. TANAKA[1], M. HORI[2], T. MAEHARA[1], S. AOKI[2];
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Abstract: *Purpose:* To investigate the microstructural damage of the brain elicited by long-standing chronic ischemia in patients with moyamoya disease, we underwent a newly developed diffusion imaging named neurite orientation dispersion and density imaging (NODDI). *Methods:* 12 patients with moyamoya disease (MMD) ranging from 19-61 yrs old (mean age, 44) and 8 normal healthy volunteers (controls) ranging from 27-64 yrs old (mean age, 44) were evaluated. Data was acquired using a 3.0-T MRI system (Magnetom Skyra; Siemens AG, Erlangen, Germany) with 3 b values (0, 700: 30axes, 2850: 60 axes) and then fitted to the NODDI model by using the NODDI Matlab Toolbox5. Created maps of orientation dispersion index (OD) and intracellular volume fraction (Vic) were fitted to standard template by SPM12. Cortical infarction, cerebral hemorrhage and the white matter lesions visible on conventional MRI and b=0 images were deleted from each map by drawing volume of interests using MRIcron. FSL was used to calculate regional values of standard atlases of each map. Two-sample t-test was performed by SPM 12 to compare maps of two groups. *Results:* By comparing the regional

values, widespread decrease in Vic was detected in the white matter of MMD compared to controls (average of all ROIs in the white matter, 0.568 vs 0.625). As for cortical regions, some areas equivalent to watershed area of anterior/posterior circulation showed lower Vic compared to controls (e.g. in 50% Juelich visual cortex V5 region, 0.430 in MMD vs 0.550 in controls). OD of white matter tended to be higher in MMD compared to controls (average of all white matter ROIs, 0.317 vs. 0.271). By whole-brain analysis with SPM, significant increase in ODI was observed in the bilateral deep white matter. *Discussion:* Recent MRI study using advanced diffusion imaging to evaluate the chronic ischemic brain structural change in patients with moyamoya disease revealed widespread decrease in parameters of the white matter compared to normal subjects. Our study's results, decreased Vic and increased OD in the white matter were also compatible with them. Unlike existing conventional diffusion images, NODDI model could be applied to the cortex as well as the white matter. By utilizing this novel characteristic, we found some cortical regions (mainly in watershed area) showed decreased Vic in patients with moyamoya disease. *Conclusion:* NODDI would be a promising tool to investigate microstructural changes caused by chronic ischemia in moyamoya disease. We would further accumulate participants, coanalyzed data with neurophysiological test and PET metabolic images, to establish the clinical significance of NODDI in moyamoya disease.

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Poster

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Support: NINDS NS078791

Title: Targeted photothombotic stroke: a method for producing upper extremity impairments in mice

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Abstract: The photothrombotic approach has become widely used as an animal model of stroke because it can be used to create small ischemic infarcts over discrete subdivisions of cortex and

can be easily combined with in vivo imaging techniques. One criticism, however, is its failure to produce a notable ischemic penumbra, the region around the ischemic core with reduced cerebral blood flow (CBF). In the standard photothrombotic method, after injection of the photooxidative dye, Rose Bengal, and laser illumination (~685nm), thrombi formation results in the simultaneous occlusion of numerous vessels in the illumination region. In the present study we combine a micromirror device (DMD) to confine green laser illumination to specific penetrating arterioles branching from the middle cerebral artery and supplying the forelimb region of motor cortex (M1) of adult male and female C57/Bl6 mice. We then assessed whether this variation of the photothrombotic approach was suitable for modeling post-stroke upper extremity impairments in mice. Prior to stroke, mice were trained on a variation of the single seed retrieval task (SSR). Seeds were randomly placed on each trial in one of three positions of varying difficulty. In order to successfully grab the seed, mice needed to change their reach trajectory on every trial. Mice were trained for ten days prior to stroke and then implanted with cranial windows over the motor cortex contralateral to their preferred reaching forelim. For vessel occlusion,. After systemic injection of either Rose Bengal for animals in the stroke condition (n=12) or saline for animals in the control condition (n=8), a 0.15-0.25mm^2 arteriole area was targeted using the DMD for 4 min of green laser illumination. Mice were then probed on their reaching performance on days 3, 5, 10, 20 and 30 following stroke. Mice in the stroke condition performed significantly worse on days 3 5 and 10 post stroke compared to control animals. However, reaching performance improved by days 20 and 30. There were significant correlations between reaching performance decrements and both the area of targeted illumination and lesion volume, as measured histologically. Thus ,this variation of the standard photothrombotic technique can be used to produce focal lesions in mouse motor cortex of varying size that cause measureable impairments in upper extremity function. Keywords: Photothrombosis, stroke, chronic upper extremity impairments

Disclosures: **T. Muhammad:** None. **T. Clark:** None. **C. Sullender:** None. **A. Tang:** None. **A. Dunn:** None. **T. Jones:** None.

Poster

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Title: Structural-functional interactions underlying mirror feedback in cortical and non-cortical stroke

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Abstract: A bilateral fronto-parietal network has been implicated for processing Mirrored Visual Feedback (MVF) when chronic stroke patients move the unaffected hand. In this study we tested dependence of MVF associated function interactions on the remaining white matter integrity within this fronto-parietal network. Subjects were stratified by lesion location to determine if functional-structural relationships underlying MVF are comparable in patients with cortical and subcortical stroke. Functional Magnetic Resonance Imaging (fMRI) and Diffusion Tensor Imaging (DTI) data were correlated to describe the relationship between mirror related functional connectivity and the structural integrity. 18 stroke subjects (10 cortical, 7F, 54±13 yrs old) performed a finger flexion task with the non-paretic hand during fMRI scanning. Visual feedback was presented in real-time virtual reality as either Veridical (same side hand) or Mirrored (opposite side hand). Dynamic Causal Modeling (DCM) was used to test modulatory interactions among regions of interest selected from the contrast between conditions. Bayesian Model Averaging (BMA) was used to determine the connection strength between regions. Fractional Anisotropy (FA), a measure of white matter integrity, was correlated with the BMA parameters of intrinsic connectivity (BMA.A) and modulatory connectivity (BMA.B) scores. In line with past results, group-level analysis revealed MVF-related activation in ipsilesional fronto-parietal regions in the absence of affected hand movement. Structural integrity (FA) of the ipsilesional parietal cortex (iPar) to ipsilesional M1 (iM1) connection was positively correlated with MVF induced modulation (BMA.B) of iPar to iM1 connectivity ($r=0.654$, $p=0.056$), and negatively correlated with modulation of connectivity from contralesional parietal cortex (cPar) to iM1 ($r=-0.713$, $p = 0.047$). Analysis comparing the cortical and sub-cortical stroke groups revealed that lesion-dependency for processing MVF may be rooted in the structural integrity of fronto-parietal connections. FA of iPar-iM1 tractography was significantly smaller in cortical stroke patients ($p=0.006$), possibly resulting in a weaker iPar-to-iM1 intrinsic modulation (BMA.A) ($p=0.051$). Collectively, these results suggest that MVF modulates iM1 through both iPar and cPar, and that cPar modulation is dominant when structural integrity between iPar-iM1 is compromised. A larger sample size in each group, and a matched healthy subject data set, would strengthen interpretations about these neural mechanisms underlying mirror feedback processing in stroke.

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Poster

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NIH T32AR047752

Title: Remote effects of stroke on brain function and motor cortex connectivity

Authors: *B. J. MCMAHAN[1], J. M. CASSIDY[2], C. O'BRIEN[1], G. BRENTLINGER[3], R. SRINIVASAN[4], S. C. CRAMER[5];
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Abstract: OBJECTIVE: Stroke is a leading cause of adult disability that occurs from compromised blood flow to the brain. Post-stroke behavioral deficits result from a combination of structural injury and functional injury, which can be remote from the stroke infarct. The objective of this study was to determine the association between measures of motor system injury and motor cortex (M1) function and connectivity in patients hospitalized with stroke.**MATERIALS AND METHODS:** Twelve individuals (62.1 ± 13.7 years; 4 females) 3-28 days post-stroke admitted to an acute rehabilitation unit completed a 3-minute bedside resting electroencephalography (EEG) recording. EEG power and coherence measures in delta (1-3 Hz) and beta (20-30 Hz) frequency ranges from ipsi- and contralesional M1 were acquired with a high dense array (256-lead) EEG system. Bilateral corticospinal tract integrity was assessed in 9 of the 12 subjects with diffusion tensor imaging. Fractional anisotropy (FA) values at the cerebral peduncle level were computed. This area was unaffected by the stroke. A physical therapist assessed motor impairment and stroke severity using the Upper-Extremity Fugl-Meyer (UEFM) exam and NIH Stroke Scale (NIHSS), respectively. Associations between EEG, FA, and clinical measures were determined using Spearman's rho values.**RESULTS:** Ipsilesional FA values were negatively associated with relative delta power in ipsilesional M1 ($\rho = -0.783$; $p = 0.012$) and delta coherence between ipsi- and contralesional M1 ($\rho = -0.933$; $p < 0.001$). A ratio of ipsi/contralesional FA values negatively correlated with NIHSS score ($\rho = -0.8938$; $p < 0.001$). No significant findings were found in the beta frequency range. **CONCLUSIONS:** These findings show how the remote effects of stroke on the motor system affect ipsilesional M1 function and its interaction with contralesional M1. Importantly, stroke infarct location did not involve M1 in any of the subjects. Significant correlations involving EEG measures in the delta frequency range may reflect early neural injury more than measures in the beta frequency range.

Expanding EEG measures to include premotor and parietal motor regions may provide further understanding of the remote effects of stroke on motor system function.

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Poster

520. Stroke Imaging and Diagnostic Studies

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Topic: C.08.Stroke

Support: AHA PreDoctoral Grant

Title: Interhemispheric morphology of diffusion properties in the brainstem provides new insight to clinical assessment of chronic ischemic stroke patients with upper limb impairment

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Abstract: PURPOSE: Ischemic damage from stroke causes disruption of neural pathways; however, less is known about the resulting changes in normal appearing white matter in individuals that experience upper limb impairment. In this study, we used Diffusion Tensor Imaging to quantify microstructural integrity changes in sub-cortical regions contralateral to the lesion and in the brainstem in stroke subjects when compared to healthy controls. Additionally, we used these imaging measurements to predict impairment by Fugl-Meyer assessment (FMA), 0 (severely impaired) - 66 (not impaired).**METHOD AND MATERIALS:** MPRAGE (1.0mm³ isotropic) and spin-echo echo-planar (SE-EPI) DTI (1.5mm³ isotropic, b=1000 s/mm², 60 directions, 9 b=0 s/mm² averages) were performed on 12 moderately to severely impaired stroke individuals (FMA 11-33) and 14 healthy age-matched controls. For stroke participants, FMA was taken to quantify upper extremity impairment. Tract-based spatial statistical (TBSS) analysis was performed using fractional anisotropy (FA), radial diffusivity (RD), and axial diffusivity (AD) measures.**RESULTS: We demonstrate widespread decreased FA and increased AD and RD in sub-cortical regions contralateral to the lesion in stroke when compared to control; however, we observed the opposite trend in the midbrain. Surprisingly, for the stroke subjects, increased FA in the midbrain significantly correlated with FMA (r=.64, p=.032). Corticospinal tract AD also negatively correlated with FMA (r=-.69, p=.02),**

suggesting that corticospinal tract and brainstem microstructural properties may be important factors for clinical outcome.CONCLUSION: Our results describe the widespread structural changes that occur post-stroke, but highlight the importance of the diffusion properties within the corticospinal tract and midbrain in predicting an individual's clinical impairment level in chronic stroke.

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Poster

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Title: Stroke infarct volume estimation: Comparison of diffusion kurtosis imaging compared to diffusion weighted imaging and histology

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Abstract: In stroke treatment, diffusion weighted MRI (DWI) is an important diagnostic tool for detection and delineation of the infarct and is widely used in treatment planning. However, improvement to these MRI techniques would further aid diagnosis and treatment. In contrast to commonly used diffusion weighted imaging (DWI) protocols, a newer DWI technique, called diffusion kurtosis imaging (DKI), takes deviation from Gaussian diffusion into account, potentially providing better microstructural sensitivity, thereby supplementing the information contained in traditional DWI diagnosis based on diffusivity estimates such as mean diffusivity

(MD). The added benefit of DKI in combination with DWI in stroke is the subject of this study where infarct volume estimates based on MD and mean tensor kurtosis (MKT) are compared to histological staining. Male Sprague Dawley rats (n=7) were subjected to transient middle cerebral artery occlusion (MCAO) for 60 min and reperfused for 24 h before perfusion fixation. The fixed brains were subjected to ex-vivo high-field MRI allowing standard DWI analysis and fast DKI estimation. Brains were sliced in 40 μm cryosections for histology (hematoxylin) for infarct volume estimation using Cavalieri estimator and 2D nucleator and immunohistochemistry (IHC) using anti-GFAP (glial fibrillary acidic protein) for visualization of morphological changes in astrocytes.No differences were seen between whole brain volume estimations by DWI and histology. Likewise, the estimated infarct volumes by MD and histology did not differ. In contrast, substantially larger infarct volumes were estimated using MKT. To investigate if the larger MKT infarcts could be correlated with microstructural changes, sections were stained with anti-GFAP. This revealed a larger area of ischemia-affected tissue than seen by histology. The anti-GFAP stained tissue was separated in three different zones: an advanced infarcted zone containing astrocytes with necrotic features, a penumbra-like-area containing astrocytes with vaguely appearing branches and non-ischemic tissue. This study shows that MD-based infarct volume estimations in perfused-fixed tissue are comparable to estimations based on histological stainings and stereology of cryosections in experimental stroke. Intriguingly, the newer MKT-based infarct volume estimations might include a zone surrounding the infarct area showing astrocyte abnormality in what is thought to be the penumbra. Further studies will elucidate whether DKI in combination with DWI can be used to estimate both the penumbra and the infarct core, and thus, give an estimate of the salvageable tissue in a clinical setting.

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Poster

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Title: Rapid EEG of acute ischemic stroke in the emergency room

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Abstract: INTRODUCTION: Treatment of acute stroke requires rapid, accurate diagnosis. An additional challenge is to identify candidates for acute reperfusion. EEG detects cerebral ischemia immediately and might have diagnostic utility in this setting. Application of EEG in the acute disease setting has historically been limited by technical limitations. These may be overcome by advances in EEG hardware and software, motivating the current study, which hypothesized that rapid EEG would be feasible and would rapidly detect acute ischemia among patients transported to an Emergency Room (ER) for possible stroke.
METHODS: Patients with suspected acute stroke seen in the UC Irvine Medical Center ER consented to a 3 min resting EEG using a 256 lead dense-array system (EGI, Eugene, OR) that uses a saline lead cap. EEG recordings were obtained in parallel with ER assessments and therapy. The two EEG metrics of interest were power in the delta (1-3Hz) frequency range and the high beta frequency range (20-30Hz), in a frontal and an occipital lead over each hemisphere.
RESULTS: EEG was obtained in 25 subjects (mean age 63 yr), 12 with radiologically confirmed acute ischemic stroke (median NIHSS=4; 4 got IV tPA); 13 were discharged with a non-stroke diagnosis such as TIA, seizure, and encephalopathy. Time from signed consent to EEG averaged 12.2 min and with practice was as short as 5 min. EEG was acquired as early as 1.6 hr after stroke onset, and was readily captured during IV tPA drip. In the C3, C4, O1, and O2 leads, delta power was 1.78-1.96 fold higher in subjects with stroke vs. non-stroke ($p<0.05$ to 0.003); no differences were significant for beta power. Findings for the ipsilesional findings (C3 and O1) were not attributable to injury to underlying cortical regions.
CONCLUSIONS: Dense array EEG can be obtained easily, rapidly, and safely in the ER acute stroke setting. Increased delta power, an established marker of brain injury, performed well in identifying stroke. Interestingly, delta power increases were widely distribute, beyond sites of injury, providing a measure of the network-wide effects resulting from focal stroke injury. This finding extended to the contralesional hemisphere--unilateral acute injury produced bilateral derangement in cortical function--indicating that EEG measures of altered brain function in the acute stroke setting provide information not apparent from structural imaging.

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Poster

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Title: Specificity of neural system injury in relation to motor and proprioceptive deficits post-stroke

Authors: *M. L. INGEMANSON[1], J. B. ROWE[2], V. CHAN[3], D. J. REINKENSMEYER[3,1,2], S. C. CRAMER[1,4];
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Abstract: *Background*: Somatosensory function is a highly distributed neural network. However, while brain regions that support proprioception are known, neural correlates of proprioceptive deficits after CNS injury remain poorly understood. Here, we aimed to understand the specificity of neural system injury in relation to motor and proprioception behavioral deficits post-stroke.
Methods: Subjects with unilateral chronic stroke were recruited. Contralesional proprioceptive function was assessed using a robot-based measure of passive finger joint position sense. Upper extremity motor function was assessed using the Fugl-Meyer Scale.
High-resolution T1-weighted and T2 FLAIR images were used to quantify motor system and sensory system injury. Each subject's infarct was outlined, and extent of overlap calculated for three gray matter regions of interest: primary motor cortex hand area (M1), primary somatosensory cortex hand area (S1), and secondary somatosensory cortex (S2). White matter injury was quantified as degree of overlap between each infarct and a canonical corticospinal tract (CST) or thalamocortical sensory tract (TST), each generated from healthy controls using diffusion tensor tractography.
To quantify total sensory system injury, S1, S2, and TST injury measures were each standardized then averaged together for each subject. For total motor system injury, standardized injury measures of M1 and CST were averaged.
Results: 27 subjects completed the study (mean age 58 yrs; Fugl-Meyer: 45 out of 66; 30 mo post-stroke). Proprioception deficits were found in 66% of subjects. There was no correlation between proprioception and motor function measured with the Fugl-Meyer score (p=0.3).

Individual injury measures for S1, S2, and TST did not significantly correlate with proprioception function. However, total sensory system injury was strongly associated with greater proprioceptive impairment (r=0.57, p=0.009). In contrast, total motor system injury and M1 injury did not correlate with motor function, but rather more severe injury to the motor tract (CST) was associated with greater motor impairment (r=0.45, p=0.04).
Conclusions: These results suggest that proprioceptive function relies on a neural network that is more distributed than that for motor function, and is less sensitive to focal tract injury than motor function. This enhanced understanding of the specific association between brain injury and proprioceptive dysfunction could lead to advances in diagnosis and treatment after stroke.

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Poster

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Title: Neural substrates supporting compromised hand function in the chronic phase of stroke

Authors: *K. P. REVILL[1], J. FREEMAN[1], G. KOWALSKI[1], M. HAUT[2], S. BELAGAJE[1], C. M. BUETEFISCH[1];
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Abstract: Ischemic stroke often impacts the integrity of primary motor cortex (M1) and its corticospinal projections (CST) resulting in incomplete recovery of hand function. The neuronal substrate supporting compromised hand function in the chronic phase of stroke is not well understood. The objective of this fMRI study is to determine the relationship between hand function and performance related activation in ipsi- and contralesional M1. Seventeen patients with chronic stroke (9M, age = 61.4 ± 12.2 years, months since stroke = 33.5 ± 35.1) participated in the study. The score on a standardized time-based hand function test (Jebsen test contrast ratio = 0.50 ± 0.2) and the mean peak acceleration recorded across five ballistic wrist extension movements were used as measures of affected hand motor and kinematic function. In a block design fMRI task, participants performed cued wrist extension movements with the affected

hand. Structural MRI images were acquired to determine lesion size and location. Following enantiomorphic normalization, BA 4 maximum probability masks were used as anatomical ROIs, masked with lesion location where appropriate. Mean percent signal change (PSC) values from each ROI were dependent variables in a regression model with age included as a covariate. Peak acceleration and Jebsen scores were negatively correlated, with higher peak acceleration predicting a lower score (less impairment) on the Jebsen test. Peak acceleration significantly predicted PSC in both ipsi- and contralesional BA 4, with reduced activation when peak acceleration was high. Higher Jebsen scores were associated with higher PSC in contralesional but not ipsilesional BA 4. Exploratory analyses (FWE-corrected) in the contralesional hemisphere showed an additional positive correlation between the Jebsen score and PSC in the cerebellum and negative correlations between peak acceleration and PSC in BA 5 and 6. Conclusions: In chronic stroke, superior hand function is associated with less activity in contralesional BA 4. Activity in contralateral BA 4 is typically low for simple motor tasks in healthy controls. Likewise, patients with good hand function do not depend on contralesional BA 4 activity when executing a simple motor task. Similarly, the negative correlation between less activity in ipsilesional BA 4 during the execution of a simple motor task and better hand kinematic function supports the notion of more effective processing of the motor command. Like measures of ipsilesional CST structure and function, ipsilesional BA 4 activity is not correlated with Jebsen score, suggesting that more complex motor tasks depend on reorganization of additional brain areas.

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Poster

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Title: High-dimensional therapeutic inference in the focally damaged human brain

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Abstract: The functional architecture of the human brain is known from neuroimaging to be highly complex yet remarkably consistent across individuals. This makes the inverse inference from brain structure to behaviour insecure when simple, low-dimensional models of the brain are used, but potentially powerful where complex, high-dimensional models are combined with large-scale data. Focusing on the consequences of acute stroke, here we use the largest reported set of anatomically registered human focal brain lesions (N=1333) to show that the low-dimensional lesion-deficit modelling implicit in conventional evaluations of focal brain damage obscures positive treatment effects. High-dimensional modelling, however, is shown to leverage complex architectural regularities in the brain to unveil substantial effects invisible to other inferential methods. The common failure to replicate in humans positive findings from animal studies may thus have a remediable inferential cause, and need not reflect physiological differences. A wholesale re-evaluation of therapeutic inference in focal human brain damage is thereby compelled.

Disclosures: **T. Xu:** None. **H.R. Jäger:** None. **M. Husain:** None. **G.E. Rees:** None. **P. Nachev:** None.

Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 520.20/JJ8

Topic: C.08.Stroke

Support: NINDS NS078791

Title: Enlarging the penumbra with a slight variation of the standard photothrombotic technique: targeted artery occlusion

Authors: *T. CLARK[1], C. SULLENDER[2], S. KAZMI[2], A. DUNN[2], T. JONES[1];
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Abstract: Photothrombotic stroke is a popular stroke model due to its ability to produce reliable, small infarcts that can be placed within distinct functional subdivisions of cortex, and its ability to be easily combined with *in vivo* imaging techniques in order to monitor stroke over time in the living brain. However, this approach has been criticized for its failure to produce a substantial ischemic penumbra, the region around the ischemic core with reduced cerebral blood flow (CBF). Here we assess the extent of the ischemic penumbra using both the traditional photothrombotic stroke approach and a variation allowing for patterned light illumination that targeted only pre-selected portions of surface arterioles. Photothrombotic lesions were

introduced by intravenous or systemic delivery of Rose Bengal followed by either patterned laser power (685nm, 20mW) over mouse motor cortex for 4 minutes, or unfocused laser power (20-24mW) for 12-15 minutes. Slight variations in the timing and delivery of Rose Bengal ensured equal lesion sizes between approaches. Cerebral blood flow was monitored with multi-exposure speckle imaging prior to and immediately following ischemia; and then again at 6 hours, 2 days, and 5 days following occlusion. Speckle imaging of cerebral blood flow (CBF) was analyzed using a custom made MATLAB script to estimate relative CBF changes from baseline. Parenchymal CBF was analyzed at each imaging time point at selected regions of interest (ROI) binned within various distances (<100um, 100-250um, 250-500um, >500um) from the ischemic core, defined as the area of parenchyma with CBF below 20% of baseline CBF at 2 days post stroke. At 2 days post occlusion, CBF in both groups was significantly reduced between 20-60% of baseline CBF within 250um of the ischemic core. However, animals that received the traditional photothrombosis showed near baseline levels of CBF at distances greater than 250um from the core whereas animals that received targeted photothrombosis showed decreased (40-80%) levels of baseline CBF even in parenchymal regions greater than 500um from the ischemic core. By 5 days, CBF in both groups returned to 60-80% of baseline blood flow within 250um of the ischemic core. However, animals that received traditional photothrombosis showed suprabaseline CBF at distances greater than 250um whereas animals that received targeted photothrombosis retained decreased (60-80%) CBF more than 500um from the core. These data demonstrate that patterned illumination during photothrombosis, creates a substantial ischemic penumbra, which makes it a more clinically relevant stroke model than the traditional photothrombotic method.
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Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

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Program#/Poster#: 520.21/JJ9

Topic: C.08.Stroke

Title: Analysis of evoked potentials during successful treatment of carotid artery disease

Authors: ***M. KELLY**[1], L. PEELING[2], K. MEGURO[2], J. NORTON[2];
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Abstract: Carotid artery stenosis is a leading cause of stroke, and therefore disability. Removing the stenosis through either open surgery or endovascular treatment has been shown to reduce the risk of subsequent stroke. Open surgical treatment involves the occlusion of the artery for the period of time in which the plaque is being removed. In endovascular surgery stents and catheters are placed into the artery and there is often a period of blood flow occlusion during angioplasty. To forewarn of impending neurological damage it is common to use somatosensory evoked potentials to assess brain function during the period of occlusion. All patients were monitored with bilateral median nerve SSEPs recorded with either a Cadwell Cascade or Medtronic NIM-Eclipse, both with the same filter and stimulation settings. Data was exported for post-hoc analysis in the Matlab environment. In this study we examined the SSEP velocity and acceleration during phases of the procedure related to both blood flow occlusion and restoration. None of the cases in the study (14 surgical and 11 endovascular) resulted in changes in the SSEP according to the traditional criteria (decrease in amplitude of 50% or increase in latency of 10%). The analysis focused on the initial slope of the cortical SSEP leading to the N20 peak. Both the open and endovascular procedure resulted in an increase in the amplitude of the SSEP (7.4% and 5.2% respectively, [$p<0.05$ in both cases, paired t-test]) compared to the baseline recordings. In open surgery the clamp time resulted in a slower velocity of the potential, whilst following the clamp release it rebounded to above baseline values. This was a short-lived event, and at the end of the procedure, although the potential was higher in amplitude, there were no differences in velocity compared to baseline. In contrast, the endovascular approach did not show any significant changes in velocity. In the absence of changes in amplitude or latency that are indicative of neural damage there are other changes in the brain function that can be detected in the evoked potential. The clinical significance of the changes is not known at present. Differences between the two procedures may influence differences in neurophysiological functions.

Disclosures: **M. Kelly:** None. **L. Peeling:** None. **K. Meguro:** None. **J. Norton:** None.

Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

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Program#/Poster#: 520.22/JJ10

Topic: C.08.Stroke

Support: NIH R01 HD068488

NIH R01 NS048281

Title: Damage to white matter bottlenecks contributes to chronic language impairments and disrupts semantic network function in patients with left middle cerebral artery stroke

Authors: *J. C. GRIFFIS[1], J. P. SZAFLARSKI[2];
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Abstract: Neuroimaging evidence indicates that the normalization of processing in pre-existing language networks enables language recovery after left hemisphere stroke. Damage to white matter (WM) connections among language areas might be expected to impede recovery by disabling long-range communication and preventing the re-integration of surviving areas into coherent networks for language processing. Indeed, chronic language impairments have been linked to WM damage near regions that may correspond to bottlenecks where fibers from multiple long-range tracts converge. However, the effects of damage to verified WM bottlenecks on long-term language outcomes and language network function in stroke patients have not been investigated.

We first validated predictions of the current model of language recovery after stroke by showing that task-driven fMRI activity in the pre-existing semantic network (identified in 43 healthy individuals) during semantic decisions predicts naming, fluency, and comprehension abilities independently of damage to the network in 43 patients with chronic post-stroke aphasia. Diffusion MRI tractography of the healthy individuals verified the presence of 2 *a priori* WM bottlenecks involving (1) callosal, arcuate, inferior fronto-occipital, and inferior longitudinal fibers in the WM underlying posterior temporal regions, and (2) anterior thalamo-cortical, uncinate, and inferior fronto-occipital fibers in the WM underlying anterior prefrontal regions. Using multiple regression to control for lesion volume effects, we found that damage to the posterior bottleneck predicts chronic deficits in fluency, naming, and comprehension, while damage to the anterior bottleneck only predicts deficits in fluency. Results from a multivariate lesion-behavior analysis corroborated these findings. Whole-brain fMRI analyses revealed that damage to the posterior bottleneck was associated with reduced task-driven activity throughout the bilateral semantic network, while damage to the anterior bottleneck was associated with reduced activity only in left inferior frontal cortex.

Our results support the current model of language recovery after stroke. They also show that damage to WM bottlenecks, specifically in the WM underlying posterior temporal regions, contribute to chronic language impairments in multiple domains and disrupts function in bilateral portions of the semantic network. Damage to WM bottlenecks may represent an under-recognized source of chronic language deficits after stroke, and may underlie previous associations between damage to the posterior temporal WM and poor prognosis in patients with post-stroke aphasia.

Disclosures: J.C. Griffis: None. **J.P. Szaflarski:** None.

Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

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Program#/Poster#: 520.23/JJ11

Topic: C.08.Stroke

Support: Beatrice Meene Haggerty Center for Research on Brain Injury and Repair in Stroke

UT Dallas Green Fellowship

Title: Visualizing reorganization of cholinergic projections from the nucleus basalis after photothrombotic stroke.

Authors: *D. M. BETZ, A. BECKER, M. P. GOLDBERG;
Neurol. and Neurotherapeutics, UT Southwestern Med. Ctr., Dallas, TX

Abstract: Stroke is a devastating event with long term functional deficits. Many stokes affect the somatosensory cortex, causing specific deficits that never fully recover. Cholinergic pathways are known to modulate plasticity in many different modalities. If the cholinergic system itself is damaged or modified by stroke, it could hold important implications for stroke recovery. We hypothesize that the cholinergic projections from the nucleus basalis (NB) undergo changes in axonal density and distribution after stroke. We examined cholinergic pathways in transgenic mice expressing EYFP-tagged channelrhodopsin in cholinergic cells. We bred these mice by crossing mice with a cre transgene under the choline acetyltransferase promoter (**B6;129S6-*Chat*$^{tm2(cre)Lowl}$/J**)with mice containing a channelrhodopsin-EYFP transgene with a floxed stop cassette under the CAG promoter (**B6;129S-*Gt(ROSA)26Sor*$^{tm32(CAG-COP4*H134R/EYFP)Hze}$/J**), both obtained from Jackson Laboratories. Mice received either sham surgery or photothrombotic stroke of the left sensorimotor cortex. Behavioral function was assessed by Rotarod testing. Mice were housed in enriched environments beginning 3 weeks prior to stroke and were sacrificed at day 3, week 2 or week 4 after stroke or sham surgery. Evolution of cholinergic projections in fixed brains was visualized by high-throughput fluorescence microscopy using either whole slide imaging of cryostat sections (Nanozoomer, Hamamatsu) or serial two-photon tomography of whole brains (TissueCyte 1000, TissueVision). Cholinergic pathways were well visualized with both imaging systems. Projection axons were especially intense within brainstem nuclei, but cholinergic cell bodies and proximal processes were also well seen within basal forebrain and cerebral cortex. NB projections were best seen in sagittal sections. Results from stroke and control mice will be presented. With the diffuse characteristics of the cholinergic pathways, we anticipate disruptions in axonal density and innervation. Understanding and modulating circuits involved in plasticity after stroke may be crucial to developing new therapeutic methods.

Disclosures: D.M. Betz: None. **A. Becker:** None. **M.P. Goldberg:** None.

Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 520.24/DP04 (Dynamic Poster)

Topic: C.08.Stroke

Support: 1 R01 NS063226-06

F31 NS084538 01

MSTP T32 GM007367 38

UL1 TR000040

Title: Wide-field optical mapping (WFOM) of neural activity and hemodynamics in models of acute and longitudinal brain disease

Authors: *H. T. ZHAO[1], D. CHOW[2], M. G. KOZBERG[3], S. H. KIM[1], M. A. SHAIK[1], A. SAXENA[4], A. DOVAS[6], E. M. C. HILLMAN[5];
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Abstract: Improvements in optical imaging and availability of model animals with genetically encoded calcium indicators have opened up new avenues to explore a variety of conditions in the brain. In particular, new methods for longitudinal wide-field brain imaging in awake behaving mice allows high-resolution, multi-modal recording of functional changes during disease development and progression. Here, we present an experimental model utilizing wide-field, high-speed optical imaging of Thy1-GCaMP mice during photothrombotic stroke to determine the second-by-second progression of acute ischemia and associated waves of ischemic depolarization. Our technique allows for simultaneous acquisition of hemoglobin oxygenation dynamics, GCaMP fluorescence and laser speckle blood flow data over the course of 2-4 hours following stroke induction. Our analysis reveals that prior to ischemia, bilateral waves of neuronal activity spontaneously traverse the cortex. Soon after ischemia, waves of depolarization can be seen spreading radially across the ischemic hemisphere. Simultaneous hemodynamic and blood flow measurements reveal a profound vasoconstriction coincident with the CSD, and allow for the analysis of neurovascular coupling during these events. These methods provide an important new platform for understanding acute and longitudinal brain disease, and can provide previously inaccessible links between neural, vascular, and behavioral deterioration and recovery, as well as responses to therapy in a wide range of disease states.

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Poster

520. Stroke Imaging and Diagnostic Studies

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Program#/Poster#: 520.25/JJ12

Topic: C.08.Stroke

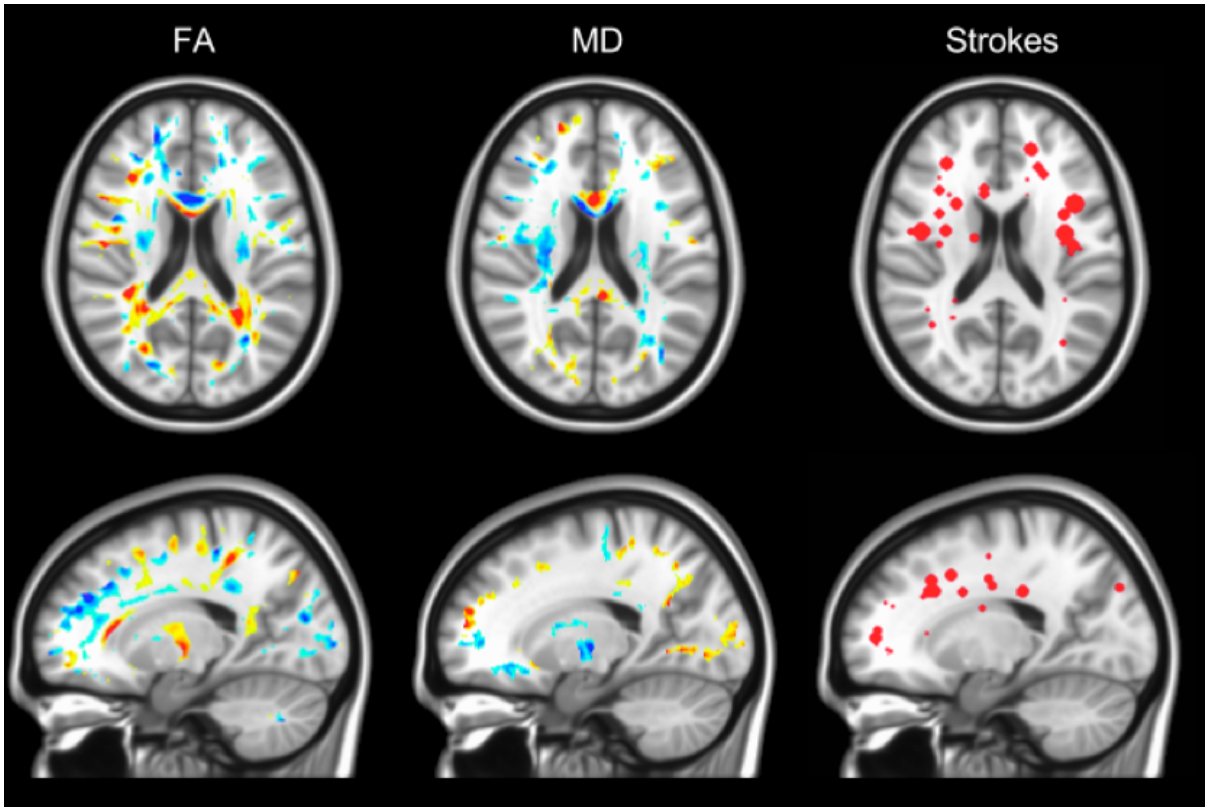
Support: NIH Grant U01HL117718

Title: DTI analysis in patients with sickle cell disease

Authors: *J. COLOIGNER[1], D. SACCHETTO[1], J. TANEDO[1], T. COATES[2], N. LEPORE[1], J. C. WOOD[3];
[1]Radiology, [2]Hematology, [3]Cardiol., Children's Hosp., Los Angeles, CA

Abstract: *Introduction* - Sickle cell disease (SCD) is a genetic disorder characterized by a mutation in the beta hemoglobin gene causing hemoglobin to polymerize upon deoxygenation, leading to chronic microvascular damage and strokes. Furthermore, recent studies have demonstrated that cognitive impairment occurs even in the absence of overt stroke on conventional imaging. We hypothesized that subtle cerebral injury might be visible with diffusion tensor imaging (DTI) in these patients.
Methods - DTI scans were obtained in 30 directions using an EPI sequence with a b-value of 1000s/mm2. 3D T1 and T2 images were collected for volumetric assessments and detection of white matter strokes. The population sample consists of 18 SCD patients and 20 healthy controls (CTL) subjects. The DTI data were processed with the Diffusion Imaging in Python (DiPy) toolbox. Images were processed to provide fractional anisotropy (FA) and mean diffusivity (MD) maps. All maps derived from the DTI images of the SCD and CTL groups were compared by paired Student's t-test. White matter strokes were found in 5 patients.
Results - Right hand figures demonstrate the cumulative white matter stroke map across all patients as a binary mask. Across frontal and parietal lobes, SCD patients had significantly higher MD and lower FA than controls. In the frontal lobe, the distribution of reduced FA matched regions of increased MD, and corresponded brain regions at high risk for stroke.
Conclusions - Although silent strokes are common in SCD, their number and location are highly variable. In contrast, our DTI data showed widespread systematic white matter abnormalities in patients with SCD in regions at risk for stroke. This suggests chronic axonal injury is occurring, even in patients who don't have overt strokes. Interestingly, regions of increased FA and decreased MD were also found in regions of the brain typically spared by silent strokes. These

may represent axonal remodeling in compensation for the frontoparietal damage. We are currently exploring the impact of these lesions on neurocognitive function.



Disclosures: **J. Cologner:** None. **D. Sacchetto:** None. **J. Tanedo:** None. **T. Coates:** None. **N. Lepore:** None. **J.C. Wood:** None.

Poster

520. Stroke Imaging and Diagnostic Studies

Location: Halls B-H

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Program#/Poster#: 520.26/JJ13

Topic: C.08.Stroke

Support: NIH/NINCHD HD065438

Title: Changes in corticospinal tract microstructure are associated with motor performance improvement in chronic stroke

Authors: *B. KIM[1], D. B. KAY[2], N. SCHWEIGHOFER[1,2], J. P. HALDAR[3,4], R. M. LEAHY[3,4], B. FISHER[1,5], C. J. WINSTEIN[1,5];
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Abstract: Background: Microstructure of corticospinal tract (CST) characterized by diffusion tensor imaging (DTI) has been shown to be a significant predictor of motor recovery after stroke in both acute and chronic stroke. While CST microstructural change during the early phase has been shown to be associated with motor recovery, there is no study showing a significant relationship between the change in CST microstructure and the change in motor performance in chronic stroke. **Purpose:** This study aims to determine if a change in ipsilesional CST (iCST) fractional anisotropy (FA) is associated with improvement in paretic upper extremity (UE) motor performance over a three-month intervention period. These data are a subset of a longitudinal Phase-I clinical trial of rehabilitation in chronic stroke (ClinicalTrials.gov ID: NCT01749358). **Methods:** Those with mild-to-moderate UE motor impairment participated (N=28, chronicity range = 0.47 to 14.38 years). MRI scans and clinical assessments were acquired at baseline and post a 3-month period. Imaging data were processed using BrainSuite14a (http://brainsuite.org/). CST tractography was reconstructed for both ipsi- and contra-lesional sides, and 3-dimensional CST masks were generated for each side. Average FA values of each voxel within CST mask was calculated for each side, and CST FA asymmetry index (FAAI) was derived. The primary motor outcome was average Wolf Motor Function Test (WMFT) log time score of distal control items. Significant changes in DTI and motor performance variables were assessed using repeated measures ANOVA. Relationship between change in DTI variables and change in motor performance was assessed using linear regression. **Results:** There was a significant decrease in WMFT log time score over a 3-month period (mean ± standard deviation of changes = -8.4±10.8 %, $p < 0.05$). Changes in the iCST FA and FAAI were not significant ($p = 0.82$ and $p = 0.15$, respectively). However, the linear regression revealed that changes in iCST FA and FAAI explained 35 % ($p < 0.0001$) and 33 % ($p < 0.01$) of the variance in change in log time score of WMFT distal items, respectively. **Discussion:** This is the first study in a chronic stroke population that has demonstrated a significant relationship between CST microstructural change and motor performance improvement. However, we did not set covariates in the linear regression, such as age and chronicity that can affect FA value, due to the small sample size. We need more studies with larger sample size to develop a better model for the relationship between brain microstructure and motor behavior.

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Poster

521. Motor Function: Compensation Stroke and Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 521.01/JJ14

Topic: C.08.Stroke

Support: NIH Grant NS084069

Title: Improving arm function in chronic stroke using myoelectric-computer interface

Authors: *E. M. MUGLER[1], S. HAMEED[1], J. E. GAIDE[1], R. D. FLINT[1], M. W. SLUTZKY[2]; [1]Neurol., [2]Neurology, Physiology, Physical Med. and Rehabil., Northwestern Univ., Chicago, IL

Abstract: Stroke can cause impaired arm function due to weakness, impaired sensation, and abnormal patterns of muscle activation. We have designed a myoelectric-computer interface (MCI) paradigm that maps activations of a pair of abnormally coupled muscles to orthogonal components of computer cursor movement. MCI users can learn to decouple these muscles by moving the cursor to targets along the mapping directions in an interactive game. In this study, we investigated improvement in arm function of chronic stroke survivors before, during, and after MCI training of 3 different muscle pairs in the upper arm over 18 sessions. We evaluated the effects of training duration and isometric vs. movement-based training conditions. We measured clinical outcome metrics as well as the degree of co-activation and arm joint kinematics during free reaching. Our first 16 subjects demonstrated improvement from baseline to the end of training and sustained improvement 4 weeks post-MCI use. Co-activation levels declined in all subjects in the targeted muscles, and elbow extension improved substantially in all subjects. Subjects showed modest improvement (Fugl-Meyer: 3.4 for 60-minute group, 3.6 for 90-minute group). Arm function, as measured by the Wolf Motor Function Test, also improved moderately (by 4.9 s and 10.3 s for 60- and 90-min groups, respectively). Arm function also improved outside of the lab (Motor Activity Log “How Often”: 4.1 and 4.9; “How Well”, 3.1 and 4.9 for the 2 groups, respectively). Spasticity decreased consistently across groups (-5.3 for 60-minute group, -4.21 for 90-minute group on MAS). Overall, longer MCI training showed improved functional outcomes. These results suggest that MCI training can reduce abnormal co-activation and spasticity and improve upper arm function in chronic stroke survivors. If successful, this paradigm could have a broad impact, and could be made into an inexpensive and portable device that many survivors could incorporate into their daily routine.

Disclosures: E.M. Mugler: None. **S. Hameed:** None. **J.E. Gaide:** None. **R.D. Flint:** None. **M.W. Slutzky:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 521.02/JJ15

Topic: C.08.Stroke

Title: Hemisphere specific motor deficits in bilateral coordination

Authors: *C. MAENZA[1,2], D. GOOD[1], R. L. SAINBURG[1,2];
[1]Neurol., Penn State Col. of Med., Hershey, PA; [2]Kinesiology, Pennsylvania State Univ., University Park, PA

Abstract: We have demonstrated hemisphere specific motor deficits in the ipsilesional arm of stroke patients that are most severe in patients with left hemisphere damage (LHD) and with severe contralesional impairment. LHD produced deficits in predictive features of coordination, initial trajectory direction, and intersegmental coordination. Right hemisphere damage (RHD) produced deficits in the ability to achieve and stabilize the final position of movement, possibly related to impedance control deficits. Stroke patients with mild to moderate contralesional impairments are able to use both arms for bilateral performance, a requisite for many activities of daily living. We hypothesize that right and left hemisphere lesions should differentially affect bilateral coordination. Specifically that LHD should produce deficits in predictive aspects of coordination, while RHD might produce deficits in feedback mediated aspects of coordination. We present a virtual object manipulation task, in which forward movement of the object trajectory depends upon lateral motions of the hands, predominantly elbow extension. Maintaining the object (bar) in midline requires the lateral trajectories, compensate one another, or are negatively correlated. Lack of such covariation produces lateral deviations in the trajectory of the virtual object. Because this task does not induce mechanical interactions between the arms, such coordination is completely dependent on neural control mechanisms. Our preliminary findings indicate that the movements of LHD patients were less coordinated than those of control participants or RHD patients in the early phase of motion. In contrast, RHD produced deficits in coordination in the later, stabilization phase of motion, but not in the early phases. Our results indicate hemisphere specific deficits in bilateral coordination.

Disclosures: C. Maenza: None. **D. Good:** None. **R.L. Sainburg:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Topic: C.08.Stroke

Support: NIH/NINDS R01NS082225

Title: Characterization of long-term gait deficits in mouse dmcao model of stroke, using the catwalk system

Authors: *E. CABALLERO-GARRIDO[1], J. PENA-PHILLIPIDES[1], Z. GALOCHKINA[2], E. ERHARDT[2], T. ROITBAK1[1];
[1]Neurosurg., [2]Dept. of Mathematics and Statistics, Univ. of New Mexico, Albuquerque, NM

Abstract: Assessment of functional deficits after experimental stroke has become increasingly important for clinical relevance of the proposed animal models or treatments. Stroke Therapy Academic Industry Roundtable (STAIR) recommends multiple endpoints (at least 2 or 3 weeks or longer after the insult) for behavioral studies in pre-clinical stroke research, to demonstrate a sustained benefit and sufficient clinical relevance. We evaluated mouse locomotion and gait impairment for four consecutive weeks after direct (distal) middle cerebral artery occlusion (dMCAO), a widely utilized model for experimental cortical ischemia. Different behavioral tests have been used to characterize somatosensory deficits after dMCAO. CatWalk test has been proposed as a sensitive tool for evaluation of the animal locomotion and gait impairment. The main goal of the present study was to provide a detailed characterization of the long-term functional recovery in dMCAO model of mouse stroke, using CatWalk automated system. C57BL/6 mice were subjected to dMCAO or sham surgeries (ten animals per group). The gait was analyzed at 7, 14, 21, and 28 days after surgery. Temporal, Spatial, Kinetics and Interpaw-coordination parameters have been assessed during the testing. Rigorous statistical analysis revealed significant differences in several parameters, including Run Duration, Average speed, and the Number of steps during the run, between dMCAO and Sham groups of animals at 7 days after dMCAO. These differences dissolved by 14 days after dMCAO. Spatial parameters including Print width, length and area, were significantly different between two groups, at 7 and 21 days after surgery. At 28 days, there were no significant differences in the above-mentioned parameters, probably due to a spontaneous post-ischemic recovery. Some of the kinetics parameters such as Body speed or Swing speed were significantly impaired at 7 days after dMCAO. The interpaw coordination parameters (such as Print positions) were significantly different between dMCAO and Sham groups at 28 days, while Stride length (for front and hind paws) was significantly affected at 7 days after dMCAO. In summary, we identified several specific gait and locomotion parameters, which are significantly affected by dMCAO-induced

brain damage at different time points after cerebral ischemia. These parameters can be used in the future studies for the assessment of the recovery after proposed treatments in dMCAO mice model of experimental ischemia.

Disclosures: E. Caballero-Garrido: None. **J. Pena-Phillipides:** None. **Z. Galochkina:** None. **E. Erhardt:** None. **T. Roitbak1:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: C.08.Stroke

Support: J. Yang & Family Foundation

Title: Electrophysiological assessment of the cervical spinal cord of the Long Evans rat following stroke injury

Authors: *L. MOORE[1,2,3], J. E. DUARTE[4], D. SCHWERZ DE LUCENA[4], H. ZHONG[5], R. R. ROY[5,6], D. J. REINKENSMEYER[4,7,8], V. R. EDGERTON[5,6], D. C. LU[2,3];
[2]Dept. of Neurosurg., [3]Neurosci. Dept., [1]Univ. of California, Los Angeles, CA; [4]Dept. of Mechanical and Aerospace Engin., Univ. of California, Irvine, CA; [5]Dept. of Integrative Biol. and Physiol., [6]Brain Res. Inst., Univ. of California, Los Angeles, CA; [7]Dept. of Biomed. Engin., [8]Dept. of Anat. and Neurobio., Univ. of California, Irvine, CA

Abstract: A stroke injury results in rewiring of long descending tracts that relay motor commands from the brain to motor pools in the spinal cord. To begin to determine whether physiological changes in the spinal cord coincide with post stroke impairments, the behavioral performance of Long Evans rats (n = 11) was evaluated in a reaching task for success rate and utilization of select forelimb muscles while the electrophysiological properties of the cervical spinal cord networks to the same forelimb muscles were evaluated using epidural stimulation. Prior to injury Long Evans rats, trained to perform a reaching task, were implanted with bilateral intramuscular electrodes in select forelimb muscles and with epidural electrodes at spinal cord levels C4, C6, and C8. Following behavioral and electrophysiological evaluation, rats received a unilateral photothrombotic stroke to the forelimb region of the sensory/motor cortex. Post injury rats showed impairments in reaching and grasping ability and reduced amplitudes in EMGs recorded from forelimb muscles involved in the task. These impairments coincided with an increase in the amplitude of evoked responses from multiple cervical spinal cord networks to the

impaired limb. The data presented here suggest that a cortical stroke affects the connectivity between the spinal cord and motor pools that activated impaired muscles.

Disclosures: **L. Moore:** None. **J.E. Duarte:** None. **D. Schwerz de Lucena:** None. **H. Zhong:** None. **R.R. Roy:** None. **D.J. Reinkensmeyer:** None. **V.R. Edgerton:** None. **D.C. Lu:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Program#/Poster#: 521.05/KK1

Topic: C.08.Stroke

Title: The effects of conditioning startling acoustic stimulus in healthy and stroke subjects - a TMS study

Authors: *Y.-T. CHEN, S. LI, P. ZHOU, S. LI;
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Abstract: Startling acoustic stimulus (SAS) is a useful tool to examine reticulospinal excitability. It is known that a conditioning SAS at 50ms prior can cause a transient suppression of transcranial magnetic stimulus (TMS)-induced motor evoked potential (MEP) at rest in healthy subjects. The induced MEP reduction was attributed to reticulo-cortical inhibition. However, it is unknown whether this phenomenon persists in stroke and during voluntary contraction. Therefore, the purpose of this study was to determine whether a conditioning SAS has different effect at rest and during voluntary contraction in healthy and stroke subjects.
TMS was delivered to the hot spot with and without a conditioning SAS (50ms prior) for left biceps in eleven healthy and non-impaired biceps in nine stroke subjects. TMS-induced MEP, TMS-induced force increment and silent period were used to determine the effect of conditioning SAS. A two-way mixed ANOVA [2 Groups (Healthy V.S. Stroke) × 2 Conditions (with V.S. without SAS)] with repeated measure on conditions was used to determine the difference between and within groups
At rest, the MEP was smaller with a conditioning SAS for both healthy (0.49 mV ± 0.37 mV V.S. 0.70 mV ± 0.55 mV, $p < 0.05$) and stroke (0.50 mV ± 0.52 mV V.S. 0.72 mV ± 0.73 mV, $p < 0.05$) subjects. During voluntary elbow flexion tasks, there were no significant differences in the induced force increment and MEP for both healthy and stroke subjects with and without a conditioning SAS. However, a conditioning SAS resulted in a significant shortening of the MEP silent period for both healthy (187.22 ms ± 22.99 ms with SAS vs. 200.56 ms ± 29.71 ms without SAS) and stroke (164.75 ms ± 22.18 ms with SAS vs. 184.63 ms ± 29.32 ms without SAS) subjects ($p < 0.05$). There were no significant Group × Condition interactions for all

parameters.
Our results showed that a conditioning SAS imposes a significant transient suppression of TMS-induced MEP at rest but not during voluntary contraction for both healthy and stroke subjects. The results of shortening of SAS effect on silent period in healthy and stroke subjects suggest that a conditioning SAS has a separate facilitatory effect on spinal motor neurons via activation of descending reticulospinal projections during sustained voluntary contraction. Similar results between stroke and healthy subjects that reticulospinal projections on the contralesional side are not likely impaired in stroke.

Disclosures: **Y. Chen:** None. **S. Li:** None. **P. Zhou:** None. **S. Li:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 521.06/KK2

Topic: C.08.Stroke

Title: Does contralesional motor cortex contribute to voluntary contraction of the impaired elbow flexors in stroke survivors

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Abstract: BACKGOUND: Reorganization of the lesioned hemisphere plays a major role in recovery after stroke, while the contribution of the contralesional motor cortex during voluntary contraction of the paretic limb is still not clear. Transcranial magnetic stimulus (TMS) to the primary motor cortex (M1) primarily induces muscle contraction and increase in force (or force increment) in the contralateral limb. The greater TMS-induced force increment in the ipsilateral limb could be indicative of contributions from the contralesional M1. Therefore, the purpose of this study was to compare the TMS-induced force increment between healthy and stroke subjects. METHODS: Eleven healthy and eight stroke subjects performed isometric elbow flexion (right side of healthy subjects and impaired side of stroke subjects) at 10%, 30% and 60% of their maximum voluntary contraction (MVC). TMS was delivered to M1 ipsilateral to the contracting biceps at rest and during three contraction tasks. TMS-induced MEP in the contralateral non-contracting biceps and TMS-induced elbow flexion force increment in the ipsilateral contracting side were quantified. Two-way ANOVA analyses were used with a between group factor (Group x2) and a within group factor (Force x3). RESULTS: For both health and stroke subjects, the contralateral MEP normalized to the MEP at rest showed force-

level dependent increase (for health: 10% MVC: 130% ± 42%, 30% MVC: 157% ± 58%, 60% MVC: 258% ± 119%; for stroke: 10% MVC: 177% ± 31%, 30% MVC: 235% ± 19%, 60% MVC: 294% ± 64%; $F(2,34)=24.81$ $p<0.05$). Furthermore, the normalized MEP is higher in stroke subjects (235% ± 18%) compared with healthy subjects *(*182% ± 15%*;* $F(1,17)=5.21$, $p<0.05$). There were no significant effects of Group or Group × Force interactions. The TMS-induced force increment (percent change from baseline force) was significantly greater in stroke subjects (8.02% ± 9.38%) than healthy subjects (1.61% ± 0.99%; $F(1,17)=5.18$, $p<0.05$). Furthermore, there was a main effect of Force. Stroke subjects exhibited higher TMS-induced elbow flexion force compared with healthy subjects only during 10% MVC contraction tasks (14.43% ± 17.75% V.S 1.01% ± 0.46%), but not during higher force levels. SUMMARY: Our findings of force level dependent increase in contralateral MEP and non-force level dependent change in the ipsilateral force increment indicate that there are bilateral M1 activations during unilateral voluntary activation of elbow flexors. However, contralesional activation is not likely contributing to voluntary force production of the impaired biceps muscles in stroke subjects.

Disclosures: **Y. Chen:** None. **S. Li:** None. **C. DiTommaso:** None. **P. Zhou:** None. **S. Li:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Program#/Poster#: 521.07/KK3

Topic: C.08.Stroke

Support: Center of Biomedical Research Excellence (1P20GM109090-01)

Title: Split belt walking increases neurovascular response during gait coordination task in stroke

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Abstract: The purpose of this project was to analyze the neurovascular responses of stroke survivors during split belt treadmill adaptation task. In this ongoing study, ten chronic stroke survivors walked on an instrumented split belt treadmill while being exposed to different belt speeds for each leg. The affected leg was on the fast/slow belt if its stride length was shorted/longer than the less affected side respectively. Functional near infra-red spectroscopy was used to measure hemodynamic changes in the cerebral cortex. We determined the changes in neurovascular responses due to immediate learning, late learning of split belt paradigm and transfer effects of split belt training to tied belt condition. Changes in mean oxyhemoglobin

values were determined from filtered data in regions of interest. There was a significant main effect of split belt condition on changes in oxyhemoglobin concentration in the supplementary motor area (p=0.007) and post central gyrus (p=0.008). Post-hoc analysis revealed significant decrease in change of oxyhemoglobin concentration from late learning to transfer stage in the supplementary motor area (p=0.033). This indicates a higher neurovascular response in our group of stroke survivors due to split belt protocol. Specifically, changes were observed in the planning and execution of the motor learning task.

Disclosures: **V. Ambati:** A. Employment/Salary (full or part-time): Biomechanics Research Building, University of Nebraska at Omaha. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Center of Biomedical Research Excellence Grant (1P20GM109090-01). **T. Rand:** None. **J. Fujan:** None. **P. Fayad:** None. **M. Mukherjee:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Program#/Poster#: 521.08/KK4

Topic: C.08.Stroke

Support: NCMRR/NICHD - Grant # HD060693

Title: An MRI-compatible, split-crank pedaling device to prevent motor compensation after stroke

Authors: ***S. M. SCHINDLER-IVENS**[1], B. D. SCHMIT[2], B. ARAND[2], B. CLELAND[3]; [1]Physical Therapy, [2]Biomed. Engin., [3]Clin. and Translational Rehabil. Hlth. Sci., Marquette Univ., Milwaukee, WI

Abstract: Motor compensation after stroke is a behavior in which the non-paretic limb performs tasks normally accomplished by the paretic limb. For example, stroke survivors with right hemiparesis often bear weight exclusively on the left leg when rising from a chair. Compensation improves independence, but it may be detrimental to motor recovery. We have preliminary data suggesting that compensation during pedaling reduces cortical activation, resulting in inferior lower limb movement. Our data also suggest that, when compensation is prevented, cortical activation increases. Thus, we suggest that rehabilitation that suppresses compensation can maximize lower limb movement via improved cortical activation. Here, we describe a novel experimental approach - an fMRI-compatible, split-crank pedaling device - that allows us to test

this hypothesis. The device is designed for pedaling during fMRI. It is fabricated from non-magnetic materials. Subjects lie supine with their feet fastened to the pedals. A crank arm connects each pedal to a crankshaft, which has a distinctive "split" design whereby the crankshaft is split into a left and right half. The two halves are fastened together with a coupler, which can be removed to eliminate the mechanical connection between pedals. Each half of the crankshaft turns an eccentric pulley that enables continuous pedaling despite no mechanical contribution from the contralateral limb. During the downstroke of pedaling, the eccentric pulley stretches an elastic band. Energy stored in the band is released during the upstroke to propel the leg back to top-dead-center. When the coupler is in place, subjects pedal with the paretic and non-paretic legs mechanically connected. Here, the legs are positioned 180° out-of-phase, as in conventional pedaling. Because the non-paretic leg is mechanically coupled to the paretic leg, it can compensate for impaired motor output of the paretic leg. When the coupler is removed, subjects are asked to pedal with both legs moving 180° out-of-phase. In this configuration, there is no mechanical connection between the right and left pedals. To pedal successfully, the paretic limb must accelerate the crank without assistance from the non-paretic leg. Moreover, the two legs must work together to maintain appropriate phasing. Coupled and uncoupled pedaling can be performed during fMRI, and measurements of position, velocity, and torque can be obtained. Consequently, we can examine the relationship between pedaling performance and cortical activation. The device has been tested in stroke (n=19) and control subjects (n=10), all of whom could pedal it after a brief familiarization.

Disclosures: S.M. Schindler-Ivens: None. **B.D. Schmit:** None. **B. Arand:** None. **B. Cleland:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 521.09/KK5

Topic: C.08.Stroke

Support: NCMRR/NICHD Grant #HD060693

Title: Split-crank pedaling reveals residual lower limb motor capacity after stroke

Authors: *B. CLELAND[1], T. GELTING[2], B. ARAND[3], J. STRUHAR[4], S. SCHINDLER-IVENS[4];
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Abstract: After stroke, compensation is a behavior whereby the non-paretic limb performs tasks normally executed by the paretic limb. For example, during pedaling the non-paretic limb produces the majority of the mechanical work. Compensation occurs because motor output from the paretic limb is abnormal. Compensation allows task performance despite poor motor output from the paretic limb, but also contributes to learned nonuse, which suppresses paretic limb use. Learned non-use is problematic because it prevents increased paretic motor output that may be possible with motor recovery. This study aimed to determine if learned non-use and residual motor capacity are present during pedaling post-stroke. To achieve this aim, we prevented compensation during pedaling with unilateral and split-crank pedaling. We hypothesized that if learned non-use and residual capacity are present, then muscle activity in the paretic limb would increase during unilateral and split-crank pedaling.

Stroke (n=19, 60 ± 10 yrs) and control subjects (n=10, 64 ± 8 years) performed conventional, unilateral, and split-crank pedaling on a custom-designed pedaling device that allows for coupled or decoupled movement of each crank arm. During conventional pedaling, the crank arms were coupled 180° out-of-phase. For unilateral pedaling, the pedals were decoupled, and subjects pedaled with each leg while the other leg relaxed. During split-crank pedaling, the pedals were decoupled and subjects attempted to simultaneously move both limbs 180° out-of-phase. Muscle activity (EMG) was recorded from the tibialis anterior (TA), medial gastrocnemius (MG), rectus femoris (RF), and biceps femoris (BF). Velocity and coefficient of variation of velocity (smoothness) were determined.

During conventional pedaling, there were no significant differences in kinematic variables between stroke and control subjects ($p = 0.602$). During unilateral and split-crank pedaling, stroke subjects increased EMG in the paretic TA, MG, and RF ($p \leq 0.001$). However, pedaling smoothness was lower in the paretic limb compared to controls during both conditions ($p < 0.001$). During split-crank pedaling, smoothness was also lower in the non-paretic limb ($p = 0.001$) and velocity was slower in both limbs ($p \leq 0.014$) compared to controls.

Our results suggest that learned non-use and residual motor capacity are present during pedaling post-stroke. Kinematic deficits that emerge when compensation is prevented might reflect remaining impairments of motor output from the paretic limb or the influence of increased demands on interlimb coordination.

Disclosures: B. Cleland: None. **T. Gelting:** None. **B. Arand:** None. **J. Struhar:** None. **S. Schindler-Ivens:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 521.10/KK6

Topic: C.09. Brain Injury and Trauma

Title: Inhibitory dysfunction in contralateral motor cortex following pediatric traumatic brain injury

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Abstract: Traumatic brain injury (TBI) affects over 1 in 5 children by 15 years of age and accounts for almost half a million emergency room visits each year. In children, TBI may be particularly problematic as they have been shown to take longer to recover and have worse outcomes after injury. Previous work from our laboratory has shown that pediatric TBI induces a preferential loss of cortical inhibition near the site of injury. However TBI patients often report symptoms related to dysfunction in brain areas contralateral to the site of injury. The consequence of TBI on the contralateral cortex remains poorly understood and is the focus of this study. To model TBI, we performed a unilateral CCI in juvenile transgenic mice with fluorescently labelled interneurons (VGAT-cre; Ai9). Following CCI we observed an overall 20% loss of GABAergic neuron density in contralateral motor cortex. Specifically, a detailed analysis of neurochemical markers of inhibitory interneurons revealed that CCI induced a 67% loss of parvalbumin (PV) expression with no change in somatostatin (SST). To assess the functional consequences of these changes we performed whole-cell patch clamp of cortical layer V fast-spiking (FS) interneurons. Layer V FS interneurons were chosen as they predominantly express PV and are known to regulate corticospinal output through axosomatic targeted inhibition. Following CCI the frequency of inhibitory synaptic events (sIPSCs) was significantly decreased by 52%. This was accompanied by a significant CCI induced increase in sIPSC charge and decay time. In contrast, the frequency of excitatory synaptic events (sPSCs) onto FS neurons was significantly increased by 55% and exhibited faster decay kinetics in CCI animals. Intrinsic properties including resting membrane potential, input resistance and firing frequency remained intact in FS neurons. These results indicate that inhibition in the contralateral motor cortex is significantly affected by a unilateral CCI in juvenile mice. The ability of TBI to disrupt the contralateral cortex may expand the consequences of brain injury well beyond the initial site of injury. The preferential loss of cortical inhibition may significantly contribute to the pathophysiology of TBI including known alterations in network excitability and disruptions in neurodevelopment.

Disclosures: **J. Nichols:** None. **J. Newbern:** None. **T.R. Anderson:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Topic: C.08.Stroke

Support: CIHR operating grant (MOP 106662)

HSF Canada Grant-in-Aid

AIHS Postgraduate Fellowship

Ontario Research Fund grant (ORF-RE 04-47)

Title: Measuring recovery of limb symmetry in subacute stroke using a robotic object hit task

Authors: *J. A. SEMRAU[1], S. H. SCOTT[2], S. P. DUKELOW[1];
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Abstract: Many everyday tasks require the use of both upper limbs. Yet some rehabilitation techniques (constraint-induced movement therapy) promote use of only the more affected limb after stroke. Little attention has focused on the relationship between the limbs throughout recovery. Recent work in our lab has developed a robotic task to evaluate bimanual function after stroke. Here we aimed to characterize the relationship of how combined use of the affected and unaffected arms evolves over the first 6 months of stroke recovery.
One hundred sixty-one subjects with first-time, unilateral stroke were evaluated at four time-points post-stroke (1, 6, 12, and 26 weeks) on a robotic object hitting (OH) task, as well as several clinical measures. For the OH task, subjects viewed both index fingers as paddles and were instructed to hit objects (red balls) away from their body that fell from the top of the screen with both hands. As the task progressed, difficulty increased, with balls falling more frequently and at a faster rate. Elapsed time for the task was approximately 3 minutes.
We found that between 1-week and 6-months post-stroke, subjects improved the average number of total hits they made (1wk: 138.6 ± 43.7 balls, 6mo: 182.8 ± 41.4), the number of hits with the affected arm (1wk: 52.14 ± 26.9, 6wk: 78.7 ± 28.6), as well as hits with the unaffected arm (1wk: 86.5 ± 25.6, 6mo: 104.0 ± 22.2). Further, when we examined the relationship between the affected and unaffected arms for movement speed and movement area throughout recovery, we found consistent increases in limb symmetry over time (R-values: Movement speed: 1wk = 0.53, 6wk = 0.54, 12wk = 0.56, 26wk = 0.65; Movement area: 1wk = 0.46, 6wk = 0.48, 12wk = 0.47, 26wk = 0.58).
Overall, we found that subjects continued to improve the number of objects hit with both the

affected and unaffected arms throughout 6 months of recovery. Further, we found that both speed and area of limb use became increasingly more symmetrical over 6 months of recovery, suggesting that when subjects are given an environment that engages limb interaction, they will utilize both the affected and unaffected limbs, despite impairment of the affected limb. This has important implications for understanding inter-limb coordination throughout stroke recovery.

Disclosures: J.A. Semrau: None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder and Chief Scientific Officer BKIN Technologies. **S.P. Dukelow:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Support: Alberta Innovates Health Solutions - Clinician Fellowship

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Heart and Stroke Foundation of Alberta, Nunavut and Northwest Territories

Alberta Innovates Health Solutions Team Grant

Ontario Research Fund Grant

Title: The inability to compensate for proprioceptive deficits using vision after stroke: a lesion analysis

Authors: *S. E. FINDLATER[1], J. A. SEMRAU[2], J. M. KENZIE[2], A. Y. YU[2], T. M. HERTER[3], S. H. SCOTT[4], S. P. DUKELOW[2];
[1]Clin. Neurosciences, [2]Univ. of Calgary, Calgary, AB, Canada; [3]Univ. of South Carolina, Columbia, SC; [4]Queen's Univ., Kingston, ON, Canada

Abstract: Background: An estimated 50-70% of stroke patients have upper limb proprioceptive impairments. Proprioception is our sense of limb position and movement. Proprioceptive impairments have been associated with incomplete upper limb recovery after stroke. Vision is widely believed to be a viable means of compensating for proprioceptive deficits. However, our group has recently demonstrated that this strategy is only successful in a small number of subjects within the first few weeks post-stroke. The aim of this study is to identify stroke lesions

that impair the ability to compensate for proprioceptive deficits using vision.
Methods: Proprioceptive deficits were quantified using a KINARM robotic exoskeleton. To assess position sense, the exoskeleton moved the subject's stroke-affected arm to one of nine spatial locations. The subject moved their unaffected arm to mirror-match that location. Subjects matched each location in a pseudo-randomized order over 6 blocks for a total of 54 trials. This task was run twice – first with a screen preventing vision of the arms and then with full view of the arms.
All subjects received anatomical neuroimaging. Lesions were marked by trained researchers, verified by a stroke neurologist, and normalized to MNI-space using SPM8. Lesion volume analysis and subtraction analysis was completed using MRIcron.
Results: One-hundred and seventy-two subjects with ischemic stroke were tested within two weeks of stroke onset. Fifty-eight percent of these subjects had right hemisphere damage and demonstrated a higher incidence (74%) of proprioceptive deficits in the no-vision task compared to those with left hemisphere damage (35%). Lesion volume was significantly greater ($p = 0.07$) in subjects who were unable to compensate for proprioceptive deficits using vision (mean 49ml ± 58) compared to those who were able to compensate (26ml ± 45) or those who didn't have deficits (10ml ± 15). Subjects unable to compensate using vision more frequently sustained damage to the occipital, parietal and sensorimotor cortices.
Seventy-nine subjects completed a follow-up assessment at 6 months post stroke. Twenty-nine of these subjects were unable to compensate with vision at the first timepoint. Of these subjects, twenty-two remained unable to compensate with vision 6 months after their stroke.
Conclusions: A significant proportion of stroke subjects are unable to compensate for proprioceptive deficits using vision. Preliminary analysis indicates that lesion hemisphere, volume and stroke location may be important considerations. These findings may provide prognostic information and guidance to rehabilitation practitioners.

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Poster

521. Motor Function: Compensation Stroke and Injury

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Topic: C.08.Stroke

Support: the NRF grant funded by the Korea government (MSIP) (NRF-2014R1A2A1A01005128)

Title: Appropriate objective assessments to predict motor function in stroke patients

Authors: *W. CHANG[1], E. PARK[1], J. LEE[1], A. LEE[1], Y.-H. KIM[1,2];
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Abstract: Introduction: Many objective assessments have used to measure the motor function in stroke patients. However, there was a lack of studies for the appropriate assessments to represent the current motor function or to predict the motor function in stroke patients. In this study, we aimed to investigate the optimal objective assessments at subacute phase to predict the motor function in stroke patients. **Methods:** Sixty-one subacute stroke patients (mean age 59.7 yrs) were recruited in this study. At 2 weeks after stroke onset, all patients took three different assessments; anatomical T1-weighted MRI to measure the stroke lesion volume, diffusion tensor imaging (DTI) to measure the preservation of anatomical corticospinal tract (CST), and transcranial magnetic stimulation (TMS)-induced motor evoked potentials (MEPs) to measure the preservation of functional CST. The upper limb scores of Fugl-Meyer assessment (FMA-UL) were measured at 2 weeks and 3 months after stroke onset. Multiple regression analysis was performed to determine the significantly independent assessments for motor function at 2 weeks and 3 months after stroke. **Results:** FMA-UL at 2 weeks and 3 months after stroke showed the significant relationship with stroke lesion volume, the preservation of functional and anatomical CST, respectively ($p<0.05$). The preservation of functional CST and the stroke lesion volume showed the significantly independent variables with motor function at 2 weeks after stroke. However, the preservation of functional and anatomical CST showed the significantly independent variables to predict motor function at 3 months after stroke. **Conclusion:** These results suggest both DTI and TMS-induced MEPs at subacute stage could be used to predict the upper extremity motor function at 3 months after stroke. In addition, the preservation of corticospinal tract at subacute stage may reveal the potentials of motor recovery in stroke patients (Supported by the NRF grant funded by the Korea government (MSIP) (NRF-2014R1A2A1A01005128)).

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Poster

521. Motor Function: Compensation Stroke and Injury

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Title: Motor unit discharge characteristics differ among proximal and distal muscles of the upper limb in healthy and post-stroke individuals

Authors: *L. M. MCPHERSON[1], F. NEGRO[2], C. K. THOMPSON[3], D. FARINA[2], C. HECKMAN[4], J. DEWALD[4];
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Abstract: Proximal and distal arm muscles serve different roles during functional tasks. Proximal muscles are typically used for postural control, and distal muscles are typically used for fine motor control. Differences in neural control of the muscles likely exist, and analysis of motor unit (MU) discharge can help elucidate them. It has not been feasible to efficiently measure MU discharge in multiple muscles until recently. High-density surface EMG is a novel approach for extracting MU discharge that provides improved efficiency and automation. Using this approach in healthy controls (N=8) and those with chronic hemiparetic stroke (N=12, moderate-to-severe impairment), we examined MU discharge characteristics (discharge rate, rate modulation) in proximal and distal arm muscles. The tested arm was placed in an isometric apparatus to measure shoulder, elbow, and finger joint torques. 64-channel EMG grids were placed on the surface of deltoid (DELT), biceps (BIC), and finger flexors (FF). Separate isometric contractions of shoulder abduction, elbow flexion, and finger flexion were performed at efforts ranging from 10 - 40% maximum torque. EMG data were decomposed into MU spike trains. Mean MU discharge rate (MDR) was calculated and compared against torque to estimate rate modulation. Differences in MU behavior were found among DELT, BIC, and FF, and the relationships between the muscles changes post-stroke. Overall MDR values were similar across muscles in controls (DELT: 13.7, BIC: 13.9, FF: 13.4 pps) but decreased from proximal to distal post-stroke (DELT: 11.4, BIC: 10.7, FF: 8.5 pps). Differences in rate modulation were also observed between groups. In controls, rate modulation increased from proximal to distal (DELT: 7.2, BIC: 16.1, FF 19.7 pps/%MVT). In stroke, however, rate modulation decreased from proximal to distal, and it was absent in finger flexors (DELT: 6.7, BIC: 4.0, FF: -0.4 pps/%MVT). Differences in rate modulation across muscles and groups may be due to differences in the balance of excitatory and inhibitory synaptic input, which significantly impacts rate modulation (Powers et al, 2012). Findings underscore the need to record from multiple muscles when using MU analysis to examine neural organization to the upper limb.

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Poster

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Title: Real time biofeedback training improves gait and plantar pressure distribution following 8 weeks of gait training in post stroke individuals.

Authors: *J. GONZALEZ PLAZOLA[1], I.-H. KHOO[2], P. MARAYONG[3], K. DEMARS[4], V. KRISHNAN[4];
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Abstract: Introduction: Asymmetry in a person's gait is a predominate factor when hemiparesis is present. The effects of asymmetrical walking patterns can be associated with irregularities in plantar pressure in the affected limb. Many commercial devices can evaluate the gait parameters relating to gait; however, using our biofeedback device, Walk-Even, a person's gait asymmetry can be both evaluated and corrected in real-time.
Methods: A total of 11 subjects, 6 males and 5 females, with chronic stroke participated in an 8-week intervention study. Six subjects were in the experimental group and the other five participants were in the control group. Both groups received strength and gait training for 40 minutes, two times per week for a total of 8-weeks. For the gait training, the experimental group was given auditory biofeedback to increase their stance time on their affected side, while the control group underwent a traditional gait training with verbal or visual cues. The subjects' average velocity, time taken to shift from heel to toe, average heel pressure, average metatarsal pressure, center of pressure (COP) trajectory, and stance and swing percentage was collected using the F-Scan device. The participants were tested before and after the intervention.
Results: Univariate ANOVA did not show any interaction, or main effect between the groups (experimental and control) and time (pre- and post-intervention) in the following variables even though the results showed a positive trend in the experimental group when compared to the control group in: COP trajectory, average velocity, average heel, average force, stance and swing percentage. Time taken to shift from heel to toe, maximal metatarsal pressure and stride time showed a significant interaction ($p<0.05$) and time effect ($p<0.05$). In addition, average metatarsal showed a significant interaction ($p<0.05$). The interaction effect in the above parameters of gait confirmed that both groups improved after 8 weeks, but the improvement was greater in the experimental group. In particular, the experimental group placed more weight onto

the forefoot during walking and also the speed of transfer was much faster than the control group.
Conclusion: This study suggests that gait training with real-time feedback using Walk-Even might reduce asymmetry while walking and increase weight-bearing on the affected side in chronic stroke survivors.

Disclosures: J. Gonzalez Plazola: None. **I. Khoo:** None. **P. Marayong:** None. **K. DeMars:** None. **V. Krishnan:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 521.16/KK12

Topic: C.08.Stroke

Support: NIH Grant R01HD053727

Title: Deficits of coordination, sensation, and strength predict motor dysfunction after stroke

Authors: *L. A. MROTEK[1,2], M. C. BENGTSON[2], T. STOECKMANN[3], C. P. GHEZ[4], J. MCGUIRE[5], R. A. SCHEIDT[2,6];
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Abstract: An unresolved question in the field of neurorehabilitation is how deficits of motor coordination, sensation, strength, and cognition impact activities of daily living after stroke. We investigated the extent to which these deficits can predict motor function.
Fourteen stroke survivors (>6 mo. post-stroke, 34-70 yr, 5 F) consented to participate; all were able to follow 2-step instructions. Subjects completed 2 lab-based experiments and a battery of clinical tests. In the 1st lab experiment, they sat comfortably with the more affected arm secured to a load cell measuring elbow flexion-extension torque. Maximal voluntary torque generation was determined. Subjects then tracked step changes in elbow torque using visual and auditory cues. In each 60 s trial, participants alternated between relaxing and creating torque (flexion or extension) at a moderate level (20% of maximal torque). From these data we computed several measures of motor control: reaction time, peak rate of torque change, torque change jerk, torque target acquisition error and torque target hold variability.
The 2nd laboratory experiment was a robotic test of proprioceptive acuity that determined the participant's threshold of movement detection. Participants grasped the handle of a 2D robotic

arm as it applied force perturbations ranging from 0 to 4 N in magnitude. Perturbation magnitude was adjusted until subjects could just begin to feel movement. This test estimates the likelihood that proprioception is intact based on the mean and variability of multiple movement detection estimations.
A licensed physical therapist administered tests of motor coordination [upper extremity Fugl-Myer motor (FMm)], sensation [upper extremity Fugl-Myer sensory (FMs)], muscle tone in the wrist, elbow, and shoulder [Modified Ashworth scale (MAS)], cognitive function [Montreal Cognitive Assessment (MoCA)], and sensorimotor function [13-item Chedoke Arm and Hand Activity Inventory (CAHAI)]. We used multilinear forward regression to determine the extent to which deficits of motor control/coordination, sensation, and cognition predict motor function, as quantified by CAHAI.
Regression found that 96% of the variance of CAHAI scores was predicted by 4 variables: the FM test (motor component), 78%; elbow proprioception [FM test (sensory component)], 8%; peak torque rate, 8%; and maximum elbow torque, 2%. These data indicate that coordination, sensation, and strength are all highly important for performing functional motor tasks and activities of daily living. Of these, coordination and sensation training may be the most impactful targets for therapeutic intervention.
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Poster

521. Motor Function: Compensation Stroke and Injury

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Program#/Poster#: 521.17/KK13

Topic: C.08.Stroke

Support: NHMRC Project Grant APP1058639

Title: Characterising post-stroke cortical plasticity in humans

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Abstract: Background: Animal models provide evidence of enhanced cortical plasticity after ischaemic stroke. Evidence for such a change in plasticity following stroke in humans is limited. The aim of this project was to examine the timeline of changes in cortical plasticity in patients following ischaemic stroke using non-invasive neurophysiological techniques.
Methodology: Patients were recruited following a first ever ischaemic stroke with motor involvement. Experimental sessions were performed at 7 days, 14 days, 21 days, 1 month, 3 months, and 6 months post stroke. A comparator group of healthy adults were tested three times over 12 months. At each session, functional recovery was assessed with the Action Research Arm Test (ARAT) and Functional Independence Measure (FIM). Transcranial Magnetic Stimulation (TMS) was applied to the lesioned M1 (stroke patients) or left M1 (control). Paired trains of continuous theta burst stimulation (cTBS) were used to induce a plastic response which was measured using single pulse TMS at baseline and at 4 time points following the second train of cTBS (5mins, 15mins, 30mins, 45mins).
Results: Thirteen stroke patients (8 male) aged 71.6 ± 16.5 and 16 healthy adults (10 male) aged 67.1 ± 6.3 years were recruited. There was a significant decrease in MEP amplitude following cTBS ($p=0.05$) in healthy control subjects that did not change between the 3 testing sessions. There was no significant response to cTBS (ANOVA, $p>0.05$) and no difference in the cTBS response over the 6 sessions (ANOVA, $p>0.14$) in the stroke patients. Using the 1-month post stroke ARAT score patients were then categorised into "excellent recovery" (n=7 (57/57 on ARAT) or "suboptimal recovery" (n=9, mean ARAT 35.2 (range 0-55)). There was a significant difference in cTBS response between testing sessions for the excellent recovery group, with a significant cTBS response at the 3-week time-point. There was no significant difference in cTBS response between sessions for the suboptimal recovery group and no significant response to cTBS on any testing occasion.
Conclusion: These results suggest that the cTBS induced plasticity response is reduced in stroke patients when compared to healthy aged matched controls. However, in contrast to the suboptimal recovery group, patients with excellent recovery at 1-month after stroke had a significant response to cTBS at 3 weeks post stroke. These preliminary data suggest greater plasticity is present in the acute phase following ischaemic stroke in patients who recover well. This novel human data is essential to understand when rehabilitation or regenerative medical therapies may best be administered to post-stroke patients.

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Poster

521. Motor Function: Compensation Stroke and Injury

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: C.08.Stroke

Support: The study is supported by the research program "Physical activity and nutrition for improvement of health" funded by the University of Copenhagen Excellence Programme for Interdisciplinary Research.

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Title: Relation of cortico-muscular coherence and finger motor performance following stroke

Authors: *L. H. LARSEN[1], I. C. ZIBRANDTSEN[2], T. W. KJAER[2], M. S. CHRISTENSEN[1], H. LANGBERG[1], J. B. NIELSEN[1], T. WIENECKE[2];
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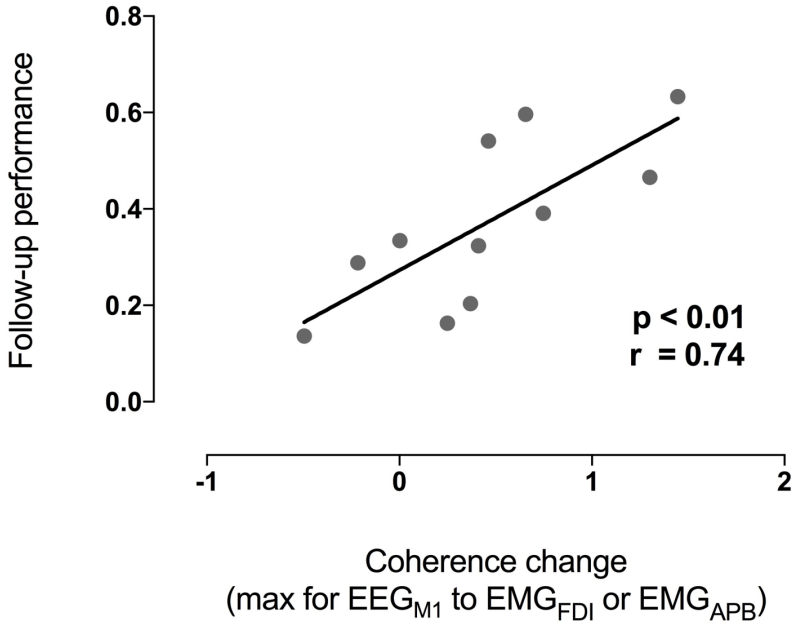
Abstract: *Objective:* Stroke is the most common cause of physical disability in the world today. It is widely assumed that damage of the motor cortex or corticospinal tract is a major cause of impaired motor function. The role of adaptations in the corticospinal drive for motor recovery is still not well understood.
Methods: Here we examined 16 patients (mean age 62 years, range 32-87 years) with clinically diagnosed stroke. The patients had unilateral mild to moderate motor impairment of the hand (8 dominant hand affected). Each patient attended two sessions at approximately 5 and 38 days post stroke. In both sessions electrophysiological recordings were obtained during three blocks of 50 precision pinch movements using the affected hand. Data recorded during movements included electroencephalographic (EEG) activity from the contralateral primary motor cortex (M1) and electromyographic (EMG) activity from first dorsal interosseous (FDI) and abductor pollicis brevis (APB) muscles in the affected hand. Changes in the corticospinal drive were evaluated from coupling in the frequency domain (coherence) between EEG and EMG.
Results: Preliminary results suggest that EEG_{M1}- EMG_{APB} coherence increases significantly in the beta band (15-30 Hz) within the first 38 days following stroke. EEG_{M1}- EMG_{FDI} coherence did not change significantly within the same period. However, the most distinct individual change in coherence was directly correlated to follow-up performance (Figure) ($p<0.01$). These results should be interpreted with caution as currently only 11 patients have been through follow-up examinations.
Conclusions: Finger motor performance 5 weeks post stroke is correlated with improvement in coherence. We propose that the changes in coherence reflect plastic changes in the corticospinal drive to the spinal motor neurons related to optimization of task performance. Individual task strategies may explain why EEG_{M1}- EMG_{FDI} coherence was not improved after 5 weeks. We

speculate that changes in the corticospinal drive may be an important part of the mechanism of motor recovery after stroke.



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Poster

521. Motor Function: Compensation Stroke and Injury

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 521.19/KK15

Topic: C.08.Stroke

Support: Johnson Center for Innovation and Translational Research

Title: A tablet-based tool for accurate measurement of hand proprioception after stroke

Authors: ***H. J. BLOCK**[1,2], J. L. MIRDAMADI[1], S. RYCKMAN[1], A. K. LYNCH[1], R. WILSON[1], D. UDAYAN[3], C. L. MASSIE[4];
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Abstract: Position sense, or proprioception, plays an important role in motor control, motor learning, and functional activities. Proprioceptive deficits following stroke have been linked to motor control impairment and recovery, although the nature of this relationship is unclear. Our understanding is in part limited by the fact that current methods of measuring proprioception in the clinical setting are subjective, imprecise, and unreliable. We recently developed a portable tablet-based apparatus to measure static proprioception in the hand using an adaptive staircase procedure. In healthy individuals, the tool demonstrated better construct validity and inter-rater and test-retest reliability compared to two common clinical tests: passive motion direction discrimination (PMDD) and position matching. An additional advantage of this method is that it assesses both proprioceptive bias and sensitivity, which may provide additional insight into the nature of proprioception deficits in clinical populations. Here, we evaluate the effectiveness of the tablet-based tool in chronic ischemic stroke survivors. We quantified proprioception at the metacarpophalangeal joint of the index finger of each hand using three methods: the tablet, PMDD, and the proprioception subsection of the Fugl-Meyer (FM). To investigate how these proprioception measures relate to tests of other sensory modalities and motor function, we also assessed primary tactile sensation (light touch, two-point discrimination), upper limb motor impairment (Fugl-Meyer Upper Extremity) and hand dexterity (Box and Block Test). We hypothesized that the tablet would better discriminate proprioception differences between the affected and unaffected hand, and that it would be more strongly correlated with hand dexterity compared to PMDD and FM. 3 chronic stroke survivors have been tested to date, all with an MRC score of 4 or 5 in the affected hand and FM Upper Extremity scores of 51, 55, and 62, indicating mild motor impairment. All had indications of impaired tactile sensation, with worse two-point discrimination on the affected side. The tablet-based tool indicated greater proprioceptive bias (n=3) and worse sensitivity (n=2) in the affected hand compared to the unaffected hand. In contrast, PMDD did not indicate any consistent pattern, and both hands scored as unimpaired in the FM test of proprioception. Overall, these preliminary data suggest that the tablet-based tool is better able to detect subtle differences in proprioception than current clinical methods.

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Poster

521. Motor Function: Compensation Stroke and Injury

Location: Halls B-H

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Topic: C.08.Stroke

Support: Brinson Foundation

Title: Alterations in spatial electromyogram patterns of hand muscles in hemiparetic stroke survivors

Authors: *G. RASOOL, B. AFSHARIPOUR, N. L. SURESH, W. Z. RYMER;
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Abstract: We investigated high-density electromyogram (EMG) patterns recorded from the first dorsal interosseous (FDI) of hemiparetic stroke survivors. We analyzed spatial EMG patterns, i.e., muscle activity maps by comparing stroke-affected and intact muscles in the contralateral hands. We hypothesized that alteration in the neuromuscular system resulting from the stroke would be evident in muscle activity maps and an analysis of these maps in spatial domain will provide us greater insight into the nature of post-stroke alterations.We recorded 64-channel (8x8) EMG data from both FDI muscles (left/right) of a mildly impaired hemiplegic chronic stroke survivor (age 59 years, Fugl-Meyer 52) and an intact subject. We also recorded isometric contraction force using a force transducer. After performing the maximum voluntarily contraction (MVC) on each side, we recorded the EMG data at different contraction levels, i.e., 5%, 10%, 20% to 60% of the MVC. The raw EMG data were bandpass filtered 20-400 Hz and root mean square (RMS) values were calculated for each channel resulting in RMS maps (8x8) for each contraction level. We compared RMS maps from opposite hands using two parameters, i.e., the Euclidean distance and the Pearson product-moment correlation.We observed greater Euclidean distance and less correlation between muscle activity maps of the stroke survivor (impaired vs. intact) as compared to the intact participant. Our statistical analysis performed using the analysis of variance revealed significant differences for the Euclidean distance $F(1,24) = 8.39$, $p=0.008$ as well as for the correlation $F(1,24) = 33.58$, $p<.001$.We found differences (quantified using the Euclidean distance and correlation) between muscle activity maps at all contraction levels for the stroke survivor. However, no such differences were observed in the intact participant.We believe that the altered neural control after brain injury and potentially later neuroplasticity resulted in substantial changes in the neural control strategy, i.e., changes in the motor unit recruitment as well as firing rates. Furthermore, the local soft tissue may also have changed substantially after the injury. The combined effect of these alterations led to the altered spatial muscle activity patterns. In future, we plan to collect data from a larger group of stroke survivors as well as intact participants. We also plan to perform textural analysis on these maps to find how individual EMG channels are changed in relation to their neighbors after the injury.

Disclosures: G. Rasool: None. **B. Afsharipour:** None. **N.L. Suresh:** None. **W.Z. Rymer:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 521.21/KK17

Topic: C.08.Stroke

Support: HRC 11-270

Title: An algorithm to predict recovery of independent ambulation after stroke

Authors: *M.-C. SMITH[1], J. W. STINEAR[2], P. A. BARBER[1], C. M. STINEAR[1];
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Abstract: Predicting the recovery of independent ambulation after stroke may help guide expectations for recovery and assist in realistic goal setting. The prediction of gait recovery has been limited to predicting whole group outcomes, or multivariable regression analyses which aim to explain the variance in recovery after stroke, but do not provide a means for predicting recovery in individual patients in a clinical setting. This study investigated a combination of clinical, neurophysiological and imaging assessments within 1 week of stroke as potential predictors for independent mobility at 12 weeks. The aim was to create an algorithm to predict which patients will recover independent ambulation. An initial cohort of 35 patients with lower limb weakness was recruited within 3 to 7 days of ischaemic or haemorrhagic stroke (16 men, mean age 70 years, 19 right hemisphere). Baseline clinical assessments were conducted at 3 days and 1 week after stroke. These included stroke severity, strength, lower limb impairment, trunk control, balance and current ambulation. In addition to clinical measures, transcranial magnetic stimulation (TMS) was used between days 5 - 7 to assess the functional integrity of the corticospinal tract (CST) by attempting to elicit a motor-evoked potential (MEP) in the affected tibialis anterior muscle. Magnetic Resonance Imaging (MRI) was conducted at 7-10 days after stroke to assess structural integrity of the CST. The primary endpoint was independent mobility at 12 weeks, defined as a Functional Ambulation Categories (FAC) score of 4 or 5. At 1 week after stroke, 16 patients had an FAC score of 0 (unable to mobilise or requiring at least 2 people to assist), 10 required assistance or supervision to mobilise (FAC 1,2,3) and 7 had already regained independent mobility (FAC 4,5). At 12 weeks, 23 were mobilising independently, 4 required assistance and 2 were unable to mobilise. Six patients were lost to follow up due to death or illness. Preliminary data analysis indicates that an algorithm based on clinical assessments alone at 1 week after stroke may predict which patients will or will not recover independent ambulation. This differs from previous work in the upper limb in which a combination of clinical assessment, TMS and MRI is recommended. A second cohort of 18 patients (8 men, mean age 70 years, 9 right hemisphere) was recruited to test the proposed algorithm. Patients in the second cohort are currently completing primary endpoint assessments.

The findings from this study are expected to provide evidence for the use of a simple, clinically useful algorithm for predicting which patients will recover independent ambulation after stroke.

Disclosures: **M. Smith:** None. **J.W. Stinear:** None. **P.A. Barber:** None. **C.M. Stinear:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 521.22/KK18

Topic: C.08.Stroke

Support: HRC Project 11/270

Title: PREP2: A refined algorithm for Predicting REcovery Potential of upper limb function after stroke

Authors: ***C. M. STINEAR**, W. D. BYBLOW, M.-C. SMITH, S. J. ACKERLEY, P. A. BARBER;
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Abstract: Independence after stroke depends largely on the recovery of motor function. Accurate prediction of motor recovery may help clinicians and patients manage expectations, set realistic rehabilitation goals, and use their resources efficiently. However, accurate prediction is difficult for individual patients. We have previously described an algorithm for predicting potential for recovery of upper limb function for individual patients after stroke. The Predicting REcovery Potential (PREP) algorithm begins with a simple assessment of paretic shoulder abduction and finger extension strength (SAFE score out of 10), followed by transcranial magnetic stimulation (TMS), and magnetic resonance imaging (MRI), as required. Patients are predicted to have potential for a Complete, Notable, Limited or None recovery of upper limb function within 12 weeks. The PREP algorithm was developed with a dataset from 40 patients with first-ever ischaemic stroke. The aim of this study was to evaluate and refine the algorithm with a larger, more heterogeneous cohort. Inclusion criteria were confirmed stroke (ischaemic or haemorrhagic), new upper limb motor symptoms, and age at least 18 years. Previous stroke, thrombolysis and thrombectomy were allowed. Exclusion criteria were cerebellar stroke, contraindications to TMS and MRI for those patients who required these tests, reduced capacity for consent, and residing out of region precluding follow-up. The PREP algorithm was used to predict recovery of upper limb function for each patient. The Action Research Arm Test was used to measure paretic upper limb function 12 weeks post-stroke. A sample of 192 patients was recruited within 3 days of stroke (106 men, mean age 72 y, 100 right hemisphere), and 157

patients completed the 12 week assessment. The algorithm made correct predictions for 80% of patients. Of those with a Complete prognosis, 26% under-achieved and were in the Notable category at 12 weeks. Of the patients with a Notable prognosis, 42% over-achieved and were in the Complete category at 12 weeks. Using this information the algorithm was then refined by combining the SAFE score with age (<80, ≥80 years) to more accurately distinguish between patients with a Complete or Notable prognosis (88% accuracy). With the revised algorithm, only patients with a SAFE score < 5 now require TMS, reducing the proportion of patients who need this test from over half, to around one third. The revised algorithm is therefore more accurate and more efficient. The implementation of the PREP algorithm in clinical practice is described, with its potential clinical and economic benefits.

Disclosures: C.M. Stinear: None. **W.D. Byblow:** None. **M. Smith:** None. **S.J. Ackerley:** None. **P.A. Barber:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Title: Somatotopic motor mapping in the internal capsule and its implication to capsular infarct modelling in rat

Authors: H. SONG[1], W. JEONG[1], E. LEE[1], R. KIM[1], J.-Y. PARK[1], M.-C. LEE[2], *H.-I. KIM[3]; [1]Dept. of Biomed. Sci. and Engineering, Inst. of Integrated Technol., Gwangju Inst. of Sci. and Technol., Gwangju, Korea, Republic of; [2]Dept. of Pathology, Chonnam Natl. Univ. Med. Sch., Gwangju, Korea, Republic of; [3]GIST, Gwangju, Korea, Republic of

Abstract: The essence in creating the motor deficit-stroke model is to destroy the part of corticospinal tract along its pathway at the target level. For creating the capsular infarct modelling, somatotopic motor mapping (SMM) of corticospinal tract is critical in the internal

capsule. In contrast to human studies, there is a lack of SMM in rodent, which leads to the absence of unanimous target for capsular infarct lesioning. In this study, we tried to define the somatotopic location of forelimb and hind limb motor tract in the internal capsule, and we compared them with the previously published targets, to provide the better stereotactic coordinates in creating capsular infarct modelling. Twenty-nine male Sprague Dawley rats were used for this study. We used the neural tracing for SMM of forelimb and hind limb in the internal capsule. We co-injected (1μl/10min) AAV-CamKII-GFP (forelimb area) and AAV-CamKII-mCherry (hind limb area) at a titer of $2x10^{12}$ (N=9). After 3 weeks of viral expressions, rats were sacrificed for histological examinations. In addition, 20 rats were subject to capsular infarct lesioning according to previously published target: 10 for lesioning in antero-inferior target (AIG) and 10 for postero-middle target (PMG) in internal capsule. Daily behavioral tests (Single Pellet Reaching Task, Beam Walking Test, Cylinder Test and Ladder Rung Walking Test) were performed preoperatively and postoperatively to determine the motor deficit following infarct lesioning. Neural tracing showed that forelimb motor tract (FMT) was traced obliquely along the axis of corticospinal tract whereas hind limb motor tract (HMT) were traced more vertically since the forelimb area is located anterior to hind limb in the motor cortex. In the internal capsule, FMT was arranged in the ventromedial portion of the internal capsule whereas HMT in ventrolateral part of internal capsule, and superior to FMT. The overlapping of FMT and HMP were observed at the junctional area and increased more in the posterior part of internal capsule. However, area of overlapping was less than 30% in anterior part and 50 % in posterior part, respectively. AIG exhibited the severe degree of motor deficits immediately following infarct lesioning, and the motor deficits were maintained up to 14 days of observation ($p<0.001$). In contrast, motor performances were not significantly changed in PMG following infarct lesioning. In conclusion, FMT and HMT are arranged in ventromedial and ventrolateral part of internal capsule, respectively and FMT was located inferior to HMT. Accordingly, infarct lesioning in these areas is considered to be ideal target for capsular infarct modelling.

Disclosures: **H. Song:** None. **W. Jeong:** None. **E. Lee:** None. **R. Kim:** None. **J. Park:** None. **M. Lee:** None. **H. Kim:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: C.08.Stroke

Support: Baden-Wuerttemberg Stiftung (ROB-1)

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La Caixa - Deutscher Akademischer Austauschdienst (DAAD)

Title: Continuous multi-joint myoelectric control of an upper-limb robotic exoskeleton in stroke patients

Authors: *N. IRASTORZA LANDA[1,2], A. SARASOLA SANZ[1], F. SHIMAN[1], E. LÓPEZ LARRAZ[1], M. SPÜLER[3], N. BIRBAUMER[1], A. RAMOS MURGUIALDAY[1,4];
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Abstract: In recent years there has been an increasing progress in the development of upper-limb multi-joint robotic exoskeletons for motor rehabilitation designed for the training of functional arm and hand combined movements. Myoelectric control constitutes an intuitive interface for such robot-aided motor rehabilitation therapies for patients with motor impairment such as stroke. Recent research succeeded at classifying residual muscle activity of stroke patients during upper-limb discrete movements suggesting that electromyographic (EMG) signals can be a promising source for the control of rehabilitation robots in these patients. The myoelectric control of robotic exoskeletons based on the classification of residual EMG can serve to provide proprioceptive feedback to the patients based on the inferred motor intention. The development of robust pattern recognition algorithms based on EMG activity from stroke patients for simultaneous multi-joint movements and suitable training protocols for this purpose are nevertheless still challenging. Unfortunately, most of the current classification systems are limited to control a single degree of freedom (DoF) at a time, which does not allow multidimensional control of a multi-DoF rehabilitation robot for carrying out simultaneous multi-joint functional movements. In this study six healthy participants (age 20-28, right-handed) and two stroke patients underwent EMG recordings from the right and affected upper- and fore-arm muscles, respectively. They performed reaching movements in different directions combined with supination and finger extension while wearing the ISMORE 7-DoF robotic exoskeleton (Tecnalia, San Sebastian, Spain). We analyzed the EMG data and tested offline a novel pattern recognition strategy towards an online myoelectric control of the exoskeleton based on parallel working feed-forward artificial neural networks (FF-ANN) for the classification of functional combined arm and hand movements. The classifiers were fed with time-domain features extracted from the EMG signals and trained with a backpropagation algorithm. Average offline

classification results using FF-ANN for the discrimination of reaching movements in different directions and six different hand movements in healthy subjects resulted in accuracies above 66%. This classification strategy is proposed and tested offline as the decoding method for the continuous myoelectric control of a multi-DoF robotic exoskeleton. Moreover, we carried out a pilot experiment with one stroke patient using the proposed method for an online myoelectric control of the multi-DOF exoskeleton in a realistic rehabilitation scenario.

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Poster

521. Motor Function: Compensation Stroke and Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 521.25/LL3

Topic: C.08.Stroke

Title: Robotic assessment of modified constraint induced movement therapy in cynomologus macaques following middle cerebral artery occlusion

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Abstract: Constraint induced movement therapy (CIMT) has emerged as a potential rehabilitation therapy for stroke. However, its efficacy at inducing recovery is unclear due to stroke heterogeneity in humans and the use of subjective neurological outcomes. Recently, studies have demonstrated the success of non-human primate (NHP) models of stroke. Findings in a NHP stroke model have the potential to translate effectively to human clinical trials because the sample population is more controlled. Additionally, robotic assessment can provide objective determination of motor deficits and recovery. The purpose of this study was to use robotics to assess the effect of modified CIMT on stroke recovery in NHPs following middle cerebral artery occlusion (MCAO).
Stroke was induced in two cynomologus macaques by 90-minute MCAO. Associated upper limb deficits were quantified using a bilateral KINARM^{TM} exoskeleton robot to measure hand, shoulder and elbow movements in the horizontal plane as sensorimotor tasks were performed. In the perturbation posture task, the NHP must keep their hand at a central target and counter mechanical loads that are applied in varying directions to quantify proprioceptive feedback for motor actions. In the centre-out reaching task, the NHP stabilizes their hand within a central target and after an unpredictable time one of eight peripheral targets lights up which the animal

must reach to. This task quantifies control mechanisms and determines range of motion.
At 2.5 years post-stroke the two MCAO and two control NHPs were trained to saturation on the tasks described above and baseline data was collected for 30 days. We noted that MCAO NHPs used compensation strategies to accomplish tasks in the paretic arm by using the unaffected limb. Modified CIMT was then employed for 30 days by restricting movement of the unaffected arm so that it could not interfere with affected arm movements. Initially, MCAO NHPs were unable to complete tasks with the affected arm but began to overcome their maladaptive learnt non-use to make successful movements without assistance from the other limb resulting in a 31.10% increase in good trials over the intervention course. CIMT lead to a 43.02% decrease in initial direction error in one MCAO NHP and a 75.40% decrease in trial competition time in the other, suggesting improvements in range of motion and efficiency of motor behaviour, respectively. These results quantify the reversal of maladaptive compensation strategies following CIMT and suggest that robotic technology can be used to assess and implement rehabilitation strategies in a NHP model of chronic stroke.

Disclosures: **M.C. Poole:** None. **J.Y. Nashed:** None. **J. LeClerc:** None. **D.J. Cook:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Program#/Poster#: 521.26/LL4

Topic: C.08.Stroke

Support: AHA #14CRP19880025

NIDRR H133P100014

NIDRR H133P100014

NIA Claude D. Pepper Older Americans Independence Center P30-AG028747

Title: Lateral balance control after stroke: A comparison of voluntary and perturbation induced stepping reactions

Authors: *V. L. GRAY, C.-L. YANG, A. OBAH, S. MCCOMBE WALLER, M. W. ROGERS; Univ. of Maryland, Baltimore, MD

Abstract: Balance stability and recovery in persons post-stroke are compromised by limitations in limb motor control which adversely affect protective stepping. After stroke, a significant number of falls occur when weight is transferred laterally with an equal and likely number of

falls from either external factors such as slips, trips and pushes to instances during voluntary movements. Therefore, understanding stepping strategies is important for reducing falls. The purpose of this study was to compare the performance of lateral voluntary steps and reactive stepping in response to lateral waist-pull perturbations in people with chronic stroke and healthy controls. Five subjects >6 months post-stroke and 5 healthy controls (age and gender matched) participated in the study. Subjects performed 6 randomly ordered lateral external waist pull perturbations trials (2 directions x 3 repetitions), and 10 choice reaction voluntary lateral steps (2 directions x 5 repetitions) in response to a light cue. Stepping characteristics were determined from an ankle marker using 3D motion analysis. The first step characteristics including step onset time, global step length, and step height were compared between stroke (paretic, non-paretic) and controls (left, right) & voluntary and reactive steps. The perturbation-induced reactive steps did not differ for the paretic (521ms) and non-paretic leg (556ms); yet, the onset times were earlier than the voluntary steps (paretic 1000ms; non-paretic 905ms). In contrast, the voluntary steps for controls had an earlier step onset (left 420ms; right 380ms) relative to the reactive steps (left 486ms; right 488ms). The reactive steps showed an increased global step length of the paretic, 54% and non-paretic leg, 21% over voluntary trials. The voluntary trials resulted in a similar global step length of the paretic and non-paretic leg, although, it was reduced by 31% compared to controls. The step height of the non-paretic limb was similar to controls, however the paretic limb step height was increased for the voluntary (25%) and reactive (29%) steps compared to controls. Compared with healthy controls, voluntary step initiation after stroke was characterized by reduced step length and longer step initiation time, suggesting difficulties with lateral balance stability. It was evident that the capacity for initiating a quicker and longer step was indicated during perturbation-induced reactive stepping. The present results can be used to lead therapeutic approaches for improving lateral balance stability through protective stepping following stroke.

Disclosures: V.L. Gray: None. **C. Yang:** None. **A. Obah:** None. **S. McCombe Waller:** None. **M.W. Rogers:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 521.27/LL5

Topic: C.08.Stroke

Title: Exploring functional outcomes and cortical plasticity following middle cerebral artery occlusion in a Non-Human Primate

Authors: *J. Y. NASHED, J. GALLIVAN, D. J. COOK, J. LECLERC;
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Abstract: Stroke is a leading cause of death and disability worldwide. There is a high degree of variability in the recovery of stroke survivors. The reasons for the disparate recovery amongst similar patients are unclear. We hypothesize that preservation of cortical areas that support functional remapping after stroke may enhance recovery. One possibility is that the preservation of local cortical areas may mediate improved functional recovery via remapping. An alternative hypothesis is that long-range compensatory connections form and mediate recovery. Here we investigate the cortical re-organization of a translatable monkey model of stroke. To examine cortical re-organization we utilized a non-human primate model of stroke. Fourteen cynomolgus macaques underwent transient 90-minute MCAO. Perfusion, Diffusion and T2-MRI images were obtained following MCAO occlusion and at 48h and 30d. Total stroke volume and regional stroke volumes were quantified in 180 regionsof the brain. Neurobehavioural outcomes were evaluated using the Non-Human Primate Stroke Scale (NHPSS). Animals were dichotomized into good (NHPSS<9, n=7) and poor recovery (NHPSS≥9, n=7) by 30d NHPSS score. Comparisons between the good and poor recovery groups showed no difference in penumbra (PWI-DWI) and stroke core volume at baseline and no difference in total stroke volume at 48h ($P>0.05$), but differences were observed at 30 days ($P<0.05$). Functional connectivity maps were generated 30 days post-stroke. Interestingly, the poor group had stronger inter- and intra-hemispheric functional connectivity as compared to the good group. This suggests that the degree of remapping in the cortex following stroke maybe dependent on the amount of salvageable tissue in the ipsilesional cortex and penumbra.

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Poster

521. Motor Function: Compensation Stroke and Injury

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Program#/Poster#: 521.28/LL6

Topic: C.08.Stroke

Support: AHA #14CRP19880025

NIDRR H133P100014

NIA P30-AG028747

NIDRR H133P100014

Title: The effects of reactive and voluntary step training on balance recovery during lateral perturbations in individuals with chronic stroke

Authors: *C.-L. YANG, S. MCCOMBE WALLER, M. W. ROGERS, V. GRAY;
Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Background: Falls are reported as high as 73% within the first 6 months after stroke. Many falls happen while transferring weight laterally. A protective stepping strategy is commonly used to recover balance during external challenges to balance control. However, current rehabilitation practices mainly focus on intentional voluntary actions to improve balance control.
Objective: The purpose of this study was to determine the effects of perturbation-induced reactive (RST) and voluntary step training (VST) on balance recovery during lateral perturbations in individuals with chronic stroke.
Methods: Twelve community dwelling individuals >6 months post-stroke were recruited. Subjects were randomized to RST or VST group. All subjects attended a 60 minute session, 3 times per week, for 6 weeks. For RST, a motorized treadmill (the Active Step™) was used to perturb standing balance in lateral, forward, and backward directions. For VST, voluntary stepping including lateral, crossover, forward, and backward steps were practiced during each training session. The primary outcome measure was the stepping performance following lateral waist-pull perturbations (24 randomly applied trials: 2 directions × 3 repetitions × 4 magnitudes). Outcome variables included first step type, step count, and the Community Balance & Mobility Scale (CBM).
Results: When pulled toward the paretic side, an increase over baseline trials of lateral (14.5 % increase) and crossover (13.6% increase) steps and a decrease of medial steps (17.4% decrease) were found in the RST group whereas no changes were found in the VST group. When pulled toward the non-paretic side, an increase of lateral steps (RST: 24.6%; VST: 31% increase) and a decrease of medial steps (RST: 14.7%; VST: 26.2% decrease) were found in both RST and VST groups. A decreased step count after training was found in RST group regardless of pull direction, and for pulls to the non-paretic side in the VST group. Significant improvements in balance and mobility as measured by CBM were observed in both RST and VST groups (P=0.018 and P=0.043 respectively).
Conclusions: Both RST and VST can modify stepping performance as shown by an increased use of more stable step types (e.g., lateral steps) and a decrease in step count in response to external lateral perturbations. However, RST appeared to be more beneficial when perturbed toward the paretic side. These findings have implications for the development of optimal rehabilitation interventions to improve balance function and prevent falls in individuals with chronic stroke.

Disclosures: C. Yang: None. **S. McCombe Waller:** None. **M.W. Rogers:** None. **V. Gray:** None.

Poster

521. Motor Function: Compensation Stroke and Injury

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Program#/Poster#: 521.29/LL7

Topic: C.08.Stroke

Support: ORSP Internal Grant, CSULB

Title: A pilot study using the walkeven biofeedback device to improve gait symmetry after a single training session in chronic stroke survivors

Authors: C. RODRIGUEZ[1], N. BALAGTAS[2], O. ROJAS[2], I.-H. KHOO[2], P. MARAYONG[1], *V. KRISHNAN[3];
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Abstract: Introduction: In chronic stroke, asymmetric gait pattern is predominant due to muscle imbalance between the affected and non-affected lower limb muscles. We used a biofeedback device, called the Walk Even, to provide real time feedback to actively correct the asymmetrical gait pattern in subjects with chronic stroke. Our pilot study showed that the subjects' gait symmetry and stance percentage on the affected limb improved after 20 minutes of single session of training. **Methods:** A total of five chronic stroke survivors (3 males and 2 females) participated in a 20 minutes of single training session. The participants in the study were at least 6 months post-stroke, and were capable of walking 10 meters without assistance. During the gait training, the Walk Even device provided auditory feedback to encourage subjects to increase their stance time on the affected side while walking. The Walk Even device collected data from pressure insoles and processed it through a microcontroller to determine the gait parameters. The targeted stance time was based on the stance time of the normal limb. Immediately before and after training, the subjects walked 8 meters to measure gait parameters such as stance percentage and asymmetric ratio. Asymmetric ratio is the ratio between stance times of the affected and the unaffected limb. A ratio of zero implies that the subject has perfect gait symmetry. **Results:** Three subjects had increased stance percentage time (10.72%, 14.4%, and 25.47%) and a decreased swing percentage time (12.17%, 14.01%, and 24.44%) on the affected leg after one session of training. Four subjects showed a decrease in asymmetric ratio (24.72%, 35.21%, 55.13%, and 68.59%). One subject improved in all of the collected measures, and was noted to have the largest change in asymmetric ratio (68.59%). **Conclusions:** A single session of the Walk Even training improved the affected limb's stance time substantially in four chronic stroke survivors. Further randomized controlled investigation is needed in order to confirm the effectiveness of the Walk Even device on a larger population of chronic stroke survivors with asymmetrical gait patterns.

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Poster

521. Motor Function: Compensation Stroke and Injury

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Program#/Poster#: 521.30/LL8

Topic: C.08.Stroke

Support: TOYOTA Motor Corporation Grant

Title: EEG phase synchrony reflects the severity of left unilateral spatial neglect after stroke

Authors: ***T. KAWANO**[1], N. HATTORI[1,2], M. HATAKENAKA[1], Y. UNO[2], K. KITAJO[2], H. YAGURA[1], H. FUJIMOTO[3], T. YOSIOKA[1], M. NAGASAKO[1], I. MIYAI[1];
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Abstract: Objective: Focal brain lesions due to stroke can impair neural network functions. By using EEG phase synchrony index (PSI), we have reported that the PSIs between the hemispheres (IH-PSI) and between the sensorimotor areas (C3C4-PSI) showed significant correlations with motor-related clinical scales in stroke patients. The aim of the current study is to investigate the association between PSIs with electrodes placed inter-parietal lobes as well as ipsilesional fronto-parietal lobes and the left unilateral spatial neglect (USN) in stroke patients. **Methods:** Forty first-ever stroke patients with right hemisphere lesions, admitted for post-acute inpatient rehabilitation, were enrolled. Twenty subjects (mean age 65.5) presenting USN were assigned to the USN group, and twenty subjects (mean age 63.0) without presenting USN were assigned to the non-USN group. All subjects signed written informed consent, and were assessed with the Mini Mental State Examination (MMSE) for general cognitive functions. Subjects in the USN group were assessed with a USN clinical scale, which consisted of cancellation tests in the Behavioral Inattention Test. We obtained 2.5 minutes of eye-closed EEG signals according to the international 10-20 system, and inter-parietal PSI (PSI between P3 and P4 electrodes) and ipsilesional intra-hemispheric PSI (average of the PSIs the following fronto-parietal electrode pairs: P4-Fp2, P4-F4 and P4-F8) were computed in three frequency bands including theta (θ) (4-7Hz), alpha (α) (8-13Hz), and beta (β) (14-30Hz). Then, we compared the PSI values between the USN and non-USN groups, and, in the USN group, we examined the correlations of the USN scale with the inter-parietal and intra-hemispheric PSIs. For the statistical analysis, Spearman's rank correlation analysis with Bonferroni correction was used.

Results: In each electrode pair, the PSI value in the USN group was significantly lower than the non-USN group. The inter-parietal PSI significantly correlated with the USN scale in the α and θ band ($p<0.01$). The ipsilesional intra-hemispheric PSI correlated with the USN scale in the θ band ($p<0.05$). No significant correlation was observed with the MMSE scores. Since these correlations were frequency and spatially selective, the inter-parietal and ipsilesional intra-hemispheric PSIs were likely to reflect the pathophysiology of USN.
Conclusion: EEG PSI can be a useful biomarker for both motor and cognitive deficits after stroke.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Program#/Poster#: 522.01/MM1

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R01 NS042291

NIH Grant R01 EB014986

Craig H. Neilsen Foundation

Title: Cellular mechanisms influencing corticospinal and sensory axonal regeneration into neural stem cell grafts after spinal cord injury

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Abstract: Injured corticospinal tract axons regenerate robustly into caudalized neural progenitor cell (NPC) grafts and form functional synaptic connections with graft-derived neurons. However, the developmental fate of grafted NPCs, and whether those differentiated graft-derived neural subtypes might influence the regeneration of host axonal projections, remain unexplored. We demonstrate that upon maturation, embryonic spinal cord NPCs grafted into the injured, adult spinal cord contain clusters of dorsal spinal cord sensory interneurons that are potent zones of exclusion for regenerating corticospinal axons, but receive dense innervation by host $CGRP^{+}$ sensory axons, reflecting the normal topographical projection patterns of these axons into distinct spinal cord laminae. Notably, these sensory neuron clusters form curved, layered structures

populated by neuronal subtypes normally present in superficial dorsal horn laminae I-III, revealing the endogenous self-assembly of spinal cord dorsal horn-like structures within dissociated NPC grafts. These findings reveal a previously unknown barrier to corticospinal axon regeneration into otherwise highly permissive neural grafts, and more generally that axons of adult central and peripheral neurons reinnervate topographically appropriate regions of newly-born neurons after spinal cord injury. Moreover, these findings demonstrate the ability of transplanted dissociated embryonic NPCs to recapitulate assembly of adult spinal cord cytoarchitecture following engraftment into the injured, adult CNS.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Leverhulme Trust

Wings for Life

ICL

Title: NOX-dependent reactive oxygen species are essential regulators of axonal regeneration

Authors: F. DE VIRGILIIS[1], A. HERVERA[1], I. PALMISANO[1], L. ZHOU[2], G. KONG[3], T. HUTSON[1], M. DANZI[4], V. LEMMON[4], J. BIXBY[4], R. BEN-TOV-PERRY[5], M. FAINZILBER[5], C. SANTOS[6], A. SHAH[6], A. SHAH[6], *S. DI GIOVANNI[7];
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Abstract: Reactive oxygen species (ROS) contribute to tissue damage and remodelling mediated by the inflammatory response after injury. Here, we show that ROS, which are believed to promote axonal dieback and degeneration after injury, are required for axonal regeneration and functional recovery after spinal injury. We found that CX_3CR1-dependent recruitment of inflammatory cells is required for ROS and NOX2 induction in the injured sciatic nerve and dorsal root ganglia. NOX2 is released from monocytes within exosomes and incorporated in injured axons via endocytosis. Next, active NOX2 is retrogradely transported in axonal

endosomes towards the cell body via an importin-ß1/dynein dependent mechanism. Endosomal NOX2 oxidizes PTEN, leading to PTEN inactivation, stimulating PI3K-pAkt signalling and regenerative outgrowth. Challenging the view that ROS are exclusively involved in nerve degeneration, we propose a novel role for ROS in axonal regeneration and recovery of function via a NOX2-PI3K-pAkt signalling pathway.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: 1R01NS54159

1R01NS076976

Title: Anatomical evidence of axonal regeneration is detected after olfactory ensheathing cell or fibroblast transplantation in spinal rats

Authors: M. A. THORNTON, R. R. KHANKAN, M. D. MEHTA, A. K. YEUNG, K. G. GRIFFIS, H. ZHONG, R. R. ROY, V. R. EDGERTON, *P. E. PHELPS;
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Abstract: Olfactory ensheathing cells (OECs) are unique glia that support axon generation and outgrowth in the olfactory system and have shown some success as a cellular transplant therapy for the recovery of sensorimotor control after spinal cord injury. A pilot study was designed in which 10 female Sprague-Dawley rats received acute transplantation of skin fibroblasts (FB, control, n=5) or OECs (n=5) after a complete mid-thoracic spinal cord transection. All rats were implanted with epidural stimulating electrodes at spinal cord levels L1 and S2 and trained to climb an inclined grid while receiving sub-threshold stimulation for 20 min., 3 times/week for 6 months. We injected the Bartha-152 (EGFP-expressing) strain of pseudorabies virus (PRV) into the soleus and/or tibialis anterior muscles 6 days before termination to identify hindlimb motor circuits and assess connectivity across the injury site. Viral transport to cholinergic somatic motor neurons and premotor interneurons was detected in 8 rats (4 FB, 4 OEC). Three rats (2 FB,

1 OEC) had evidence of viral labeling rostral to the transection site (T3-T7), including cholinergic and Chx10-positive V2a interneurons. Serotonergic axons crossed from the rostral to the caudal stump on GFAP-positive astrocyte bridges in 2 of the 3 rats with evidence of PRV-EGFP labeling above the injury site (1 FB, 1 OEC). Together these data imply that long-term axonal regeneration occurred in two of our complete spinal rats after epidural stimulation, climb training, and olfactory ensheathing cell or fibroblast transplantation.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH Grant NS069765

Craig Neilsen Foundation

Title: A combinatorial repair strategy to promote regeneration and improve urinary function in rats with chronic thoracic contusive spinal cord injury

Authors: C.-Y. LIN[1], K. LI[1], J. SILVER[3], *Y.-S. LEE[2];
[2]Neurosci, [1]Cleveland Clin., Cleveland, OH; [3]Case Western Reserve Univ., Cleveland, OH

Abstract: In our previous studies, we have demonstrated the long distance functional regeneration of supraspinal axons in acute SCI in adult rats using a spinal cord bridging technique that incorporates peripheral nerve grafts, acidic fibroblast growth factor (aFGF) and chondroitinase ABC (ChABC). However, there is no effective treatment to promote robust nerve regeneration to restore function after chronic SCI, which is a major unmet goal. Intracellular sigma peptide (ISP) which blocks the CSPG receptor RPTPs has been shown to dramatically enhance serotonergic fiber sprouting and improve functional recovery after acute contusive SCI. Here, we investigated the effects of combining ISP with our bridging technique in facilitating nerve regeneration and bladder control after chronic SCI. Adult rats were divided into 5 groups: (1) Sham control, (2) T8 contusive SCI+PNG+ISP (3) T8 contusive SCI+aFGF+ChABC+ISP (4) T8 contusive SCI+PNG+aFGF+ChABC and (5) T8 contusive SCI+PNG+aFGF+ChABC+ISP. ISP treatment (subcutaneous injection) started 8 weeks after the original SCI for 7 weeks. The

bridging treatment was performed at 10 weeks after the original SCI. The observation period was 6 months after the original SCI. The PNG+aFGF+ChABC+ISP group showed improvements in urodynamic patterns, significantly lower bladder weights and healthier bladder morphology than the other groups. Re-lesion of the spinal cord above the injury site abrogated the improvements in urodynamic patterns in the PNG+aFGF+ChABC+ISP group. Our anatomical analyses revealed regeneration of serotonergic fibers into the PNG and also beyond the caudal end of PNG-host interface only in the PNG+aFGF+ChABC+ISP group. The intensity of serotonergic fibers at the lumbar level of the PNG+aFGF+ChABC+ISP group was significantly higher than the other SCI groups. In addition, the serotonin transporter and presynaptic markers were identified in regenerated serotonergic fibers indicating the likely functionality of these fibers. Thus, the combinatorial treatment of ISP with our bridging technique leads to induced regeneration and/or sprouting of serotonergic (and possibly other) fibers that may play an important role in improving bladder function after chronic contusive SCI.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: CNRS, INSERM, UPMC

Title: The microtubule associated protein 1b is required to overcome myelin inhibition of axon regeneration in the adult central nervous system

Authors: *F. NOTHIAS[1,2], C. BOUQUET[1], L. VINCENSINI[1,2], M.-N. BENASSY[1,2], M. VERON-RAVAILLE[1], S. SOARES[1,2];
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Abstract: Unsuccessful axonal regeneration in the central nervous system (CNS) is closely linked to the non-permissive environment, which is mainly due to reactive glial cells that expose inhibitory signals such as extracellular matrix proteins, myelin-associated proteins, but also due to poor trophic support. However, even if a favorable environment is provided, axon regeneration in mammalian CNS remains partial. Cell-autonomous factors also play a substantial role in determining the capacity for axonal regeneration, and several signaling pathways involving molecules such as cAMP, mTOR, and PTEN have been shown to be involved. Current

attempts to elicit axonal regeneration in the injured spinal cord include interference with extrinsic growth-inhibitory factors present in CNS myelin and scar tissue, their receptors, the implicated signaling pathways, and more recently, by targeting the cytoskeleton, onto which signaling pathways converge. Particularly and according to our present study, we have largely demonstrated in vitro and in vivo that MAP1B, a “juvenile” microtubule-associated protein, plays a key role in integrating signals that lead to cytoskeleton remodeling and determine axonal fate. Inhibition of axon growth by myelin components depends on neuron intracellular concentration of cAMP: embryonic neurons, with high levels of cAMP overcome myelin inhibition, contrarily to adult neurons with low levels of cAMP. Here, we investigated whether MAP1B is required for cAMP-induced axonal regeneration in a non permissive environment. In vivo, prior to a dorsal column injury on adult wildtype (wt) and MAP1B-ko mice, we performed a conditioning lesion through crush of sciatic nerve that results in increased cAMP levels in axotomized DRG neurons. Under such conditioning lesion, central projections of DRG neurons were able to re-grow across the dorsal column injury site in wt mice spinal cord, while axon regeneration was not stimulated in MAP1B-ko mice. We then compared the neurite regeneration capacity in vitro of wt- vs MAPB-ko DRG neurons plated on adult myelin. Regeneration of wt DRG neurites is poor, but significantly enhanced when cAMP is added to the medium. In MAP1B-ko DRG, however, increased cAMP levels do not promote neurite growth. These data strongly suggest that MAP1B is a key player required to overcome myelin-induced inhibition of axon regeneration in the adult CNS. We are currently investigating whether MAP1B promotes axon regeneration by modulating microtubule stability, since we have recently shown that the latter is affected by MAP1B phosphorylation.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Program#/Poster#: 522.06/MM6

Topic: C.09. Brain Injury and Trauma

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The Miami Project to Cure Paralysis

The Buoniconti Fund

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Title: A combinatorial approach to CNS regeneration using activated transcription factors

Authors: *S. MEHTA, J. L. BIXBY, V. P. LEMMON;
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Abstract: Axonal regeneration after spinal cord injury (SCI) is inhibited by neuronal intrinsic growth limitations as well as external factors. One major intrinsic limitation on axon regeneration is the altered expression and activation state of regeneration-associated transcription factors (TFs) in mature neurons of the central nervous system (CNS). In contrast, axons in the peripheral nervous system (PNS) may regenerate long distances following injury. TFs that play roles in successful PNS regeneration such as cJun, Smad1, and Stat3 have been shown to promote limited axon regeneration in the CNS. Additionally, the activities of TFs can be boosted by the addition of transcriptional activation domains such as the viral activation domain VP16. This strategy has been used with TFs Klf7 and CREB to improve CNS regeneration *in vivo*. Furthermore, previous findings from our lab show that, at least in vitro, overexpression of two or more TFs can significantly enhance neurite outgrown in CNS neuron*s*. Despite these experimental advances, the number of axons that regenerate past CNS injury sites remains low. Moreover, functional recovery is elusive; few axons remake connections to their original targets. We hypothesize that combined expression of modified TFs may serve to promote more effective CNS regeneration after injury. To test this hypothesis, we selected four TFs with vital roles in PNS regeneration - Stat3, Smad1, cJun, and Stat6 - and one developmentally downregulated TF - Klf7. We generated various “activated” forms of these TFs using exogenous activation domains and constitutively active mutants, and determined which modified TFs were best able to neurite outgrowth in cultured postnatal cortical neurons. Finally, we tested the modified TFs in combinations of twos, threes, fours, and five to find the combination that most substantially promotes neurite outgrowth in cortical neurons. Although dual combinations of these modified TFs did not appear more effective than single TFs in vitro, we found that overexpression of several 3-TF combinations promoted neurite growth more strongly than overexpression of any single TF.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Support: Wings for Life Spinal Cord Research Foundation, WFL-US-025/14

Kentucky Spinal Cord Injury Research Center (KSCIRC)

Title: Evaluating the effect of targeting ryanodine and IP3 receptors on axonal dieback following laser-induced spinal cord injury

Authors: *B. OREM[1,2], G. NALLY[1,3,2], S. BRYANT[1,3,2], D. P. STIRLING[1,3,2,4];
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Abstract: Axons of the central nervous system fail to regenerate following spinal cord injury but instead retract or dieback away from the injury site. The precise mechanisms underlying acute axonal dieback remain poorly understood. Here we investigate the role of the axoplasmic reticulum, a major calcium store within axons, in mediating acute axonal dieback. To observe axons undergoing dieback in real time we use a laser-induced spinal cord injury model. Briefly, the spinal cords are isolated from transgenic mice that express yellow fluorescent protein in axons of the Gracile fasiculus, perfused in artificial cerebrospinal fluid (aCSF), and ablated using an 800 nm laser while being imaged continuously using two-photon excitation microscopy in real-time. We chose to target two different types of receptors in the axons by introducing the drugs ryanodine or 2-APB to measure their effect on axonal dieback. Either drug was dissolved in the aCSF 1 hour post injury and continuously perfused for the remainder of the imaging session. The transected axons retracted both distal and proximal from the lesion as expected. Using Dunn's method for multiple pairwise comparison, there was no significant difference in dieback length at 5 mins or 2 hours post injury, $p > 0.05$ for both. We did find a significant difference in dieback lengths beginning at 4 hours post injury with distal axonal dieback being larger. At 6 hour post injury, the distal axons (n=800) still had significantly larger axonal dieback compared to proximal axons (n=924, $p < 0.001$). We found that targeting ryanodine receptors with ryanodine significantly reduced proximal ($p < 0.001$) and distal axonal dieback ($p < 0.001$) compared to controls at 6 hour post injury. In contrast, targeting IP3 receptors with 2-APB failed to reduce axonal dieback, but rather increased dieback lengths distal to the lesion compared to control (n=207 with 2-APB, n=215 for control, $p <0.001$). Together, these results suggest that targeting ryanodine receptors may reduce axonal dieback following SCI.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: NHLBI DIR

Title: Identification of a novel binding domain for heparin in RPTPα, but not LAR or RPTPδ: Implications for proteoglycan signaling

Authors: Y. KATAGIRI[1], A. A. MORGAN[2], N. J. BANGAYAN[2], R. JUNKA[2], H. NAGASE[2], *P. YU[3], H. M. GELLER[2];
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Abstract: Type IIa Receptor protein tyrosine phosphatases (RPTPs) have been shown to modulate neural regeneration and development. All members of this family (RPTPσ, RPTPδ, and LAR) have a cell adhesion molecule-like extracellular domain that includes three N-terminal Ig domains and four to nine fibronectin type III (FNIII) domains, as well as tandem intracellular tyrosine phosphatase domains. Compelling evidence suggests that both heparan sulfate (HS) and chondroitin sulfate (CS) are the ligands for RPTPσ and LAR, and the Lys-loop located in the first Ig domain is responsible for the ligand binding. We now demonstrate that all RPTP type IIa members display high affinity binding for heparin, CS-E, and dermatan sulfate (DS) with similar nanomolar dissociation constants. However, the reason for the different functional outputs of HS and CS binding to RPTPσ (respectively promotion and inhibition of axonal growth) remains a mystery. Although attributed to the differential oligomeric state of RPTPσ upon binding to HS, but not to CS, our data indicate that both HS and CS can cluster RPTPσ. Furthermore, we demonstrate a differential contribution of FNIII domains required for high affinity binding to glycosaminoglycans (GAGs). In particular, binding of PTPσ to heparin was not completely abolished by disruption of the Lys-loop. This, along with a decrease in binding with deletion of the fourth FNIII domain, leads us to hypothesize that there is a greater contribution of the FNIII domain in binding to GAGs than previously believed. In addition, the fourth FNIII-containing domain alone binds to heparin independently of the three Ig domains and does not bind to CS-E or DS. Thus, we speculate that this novel binding domain specific for HS on RPTPσ plays an important role in controlling the different biological actions of HS and CS.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: R01-NS092876

SHC-85400

SHC-85220

SHC-84293

Title: ChABC treatment changes the expression of PTPσ and Akt after spinal cord injury in lamprey

Authors: ***J. HU**[1], W. RODEMER[1], G. ZHANG[1], S. LI[1,2], M. E. SELZER[1,3];
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Abstract: Paralysis following spinal cord injury (SCI) is due to axon interruption and failure of regeneration. Removal of chondroitin sulfate proteoglycans (CSPGs) with chondroitinase ABC (ChABC) enhances axon growth and functional recovery after SCI, but in mammalian partial injury models, it is not clear whether this involves true regeneration of injured axons (as opposed to collateral sprouting by spared axons), nor how this treatment affects the expression of CSPG receptors and their downstream signalling pathways. To get around the limitations of mammalian models, we used complete transection of lamprey spinal cord to assess the effect of ChABC on regeneration of spinal-projecting neurons, and on retrograde neuronal death. Using the fluorescence-labelled inhibitor of caspases (FLICA) method, activity was seen primarily in the "bad-regenerating" identified reticulospinal neurons. The number of all neurons showing caspase activity increased significantly at 2, 4 and 8 weeks post-TX, compared to untransected controls. ChABC application to a fresh TX site reduced the number of caspase-positive reticulospinal neurons significantly at 2 weeks. ChABC also greatly enhanced axon regeneration at 7 and 10 weeks post-TX. Correspondingly, PTPσ mRNA expression in the perikaryon was reduced significantly, suggesting that PTPσ is involved in signalling retrograde neuronal death. PTPσ mRNA expression was positively correlated with caspase activation in neurons, and inversely correlated with regeneration of their axons at 10 weeks post-TX. We also found that Akt activation (pAkt-T308) was greatly elevated after ChABC treatment. Thus, ChABC may both enhance axon regeneration after SCI, and protect neurons from delayed retrograde death by reducing PTPσ expression and enhancing Akt activity. **Keyword:** Neuronal Death, FLICA, SCI, ChABC, PTPσ, Akt, Axon Regeneration, Lamprey **Acknowledgements:** Supported by grants R01-NS092876 (NIH, **M.E. Selzer PI**), SHC-85400 (Shriners Research Foundation, **M.E. Selzer, PI**), SHC-85220, (Shriners Research Foundation, **M.E. Selzer, PI**), SHC-84293, (Shriners Research Foundation, **J. Hu, PI**)

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Title: Combinatory treatment with hepatocyte growth factor and neural progenitor cells reduces astrogliosis and pro-inflammatory microglial activation *In vitro*

Authors: *R. DRAGAS[1,2], A. SIDDIQUI[1], M. CHAMANKHAH[1], J. HONG[1,2], M. KHAZAEI[1], M. G. FEHLINGS[1,2,3];
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Abstract: Reactive astrogliosis and neuroinflammation adversely contribute to the hostile microenvironment present after spinal cord injury (SCI), posing significant challenges to regeneration and cell transplantation strategies. Hepatocyte growth factor (HGF) is a multifunctional cytokine with immunomodulatory and regenerative capabilities that may compliment neural progenitor cell (NPC)-mediated trophic support. We hypothesize that combinatorial treatment of NPC conditioned media with HGF will result in a synergistic reduction of astrogliosis and neuroinflammation after SCI. Female Wistar rats were subjected to clip-compression SCI at C5-6 or T6-7 levels. Temporal RNA-seq was used to quantify HGF expression 3-56 days post-injury. Primary rat astrocytes were activated using TGFβ1 (10 ng/mL) and a rat-derived microglial cell line was treated with LPS (1 μg/mL) to induce M1 pro-inflammatory activation. Cells were treated with either HGF protein (10, 20, or 50 ng/mL), rat NPC 4 day conditioned media (rNPC-CM), or both HGF and rNPC-CM for 24 hours. Immunocytochemistry (ICC) and qPCR were used to assess M1/M2 activation state. ICC and Western Blot were used to assess astrocytic GFAP and CSPG production. RNA-seq revealed no significant change in endogenous HGF expression up to 56 days after cervical or thoracic SCI. Combinatorial treatment, as well as HGF alone (50ng/mL), reduced CSPG expression in reactive astrocytes. HGF alone (50ng/mL) also showed a reduction in GFAP expression. Combinatorial treatment resulted in reduced NOS-2 (M1 pro-inflammatory) and increased Arg1 (M2 anti-inflammatory) expression after M1 microglial activation. For the first time, we have shown reduced CSPG deposition, astrogliosis, and pro-inflammatory microglia with HGF-rNPC-CM combinational treatment. These findings have profound translational implications as astrogliosis and neuroinflammation are largely detrimental to stem cell therapeutic efficacy and recovery after SCI and other central nervous system injuries.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Support: NRF-2012R1A1A2007597

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2014M3A9B6034224

Title: Insulin-like growth factor-1 receptor signaling involve the survival & migration of grafted neural stem cells in the injured spinal cord

Authors: *D. HWANG[1], H. SHIN[1], H. SUH-KIM[2], B. G. KIM[1,3];
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Abstract: Therapeutic impact of neural stem cell (NSC) transplantation for spinal cord injury is constrained by poor survival and migration ability of grafted NSCs. We have previously demonstrated that survival of NSC grafts rapidly declines after transplantation in the lesioned spinal cord and that combined treadmill locomotor training (TMT) increased the NSC survival in part via insulin-like growth factor-1 (IGF-1) signaling. The current study aimed to provide genetic evidence that IGF-1 receptor (IGF-1R) in NSCs may critically regulate graft survival following transplantation into the injured spinal cord in mice. Neurospheres obtained from E14 IGF-1R (+/-) mice showed size and proliferation rate similar to those from littermate wild type (WT) embryos. However, IGF-1R (+/-) NSCs were more susceptible to cellular stresses induced by reactive oxygen or nitrogen species than those from WT embryos. The survival of IGF-1R (+/-) NSC grafts was significantly attenuated after transplantation into lesioned spinal cord when compared WT NSC grafts. TMT enhanced the extent of NSC migration from the injection site allowing more intimate contacts with host spinal cord circuitry. However, most NSCs derived from IGF-1R (+/-) mice were confined around the injection site regardless of TMT. We also observed that the IGF-1R (+/+) NSCs were highly motile with frequent formation of lamellipodia or filopodia during culture. This motility and migration ability was markedly decreased in IGF-1R (+/-) NSCs. Combination of TMT and WT NSC transplantation enhanced behavioral recovery. This TMT effect was not observed when IGF-1R (+/-) NSCs were grafted. These results indicate that the IGF-1 receptor signaling may play a central role in regulation of the survival and migration of NSC grafts after SCI.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Title: Effects on neural progenitor cell grafts on neuropathic pain outcomes in models of spinal cord injury

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Abstract: Neural progenitor cells (NPCs) grafted into sites of spinal cord injury (SCI) survive and differentiate into neural cells, including neurons that extend axons in very large numbers over long distances, resulting in improved motor functional outcomes (Lu et al, 2012; Kadoya et al, 2016). An important step in the clinical translation of NPC therapies for use in the treatment of SCI is to evaluate their impact on neuropathic pain outcomes in models of SCI, particularly as there have been previous reports of stem cell therapies for SCI generating neuropathic pain (Hoftetter et al., 2005). To address this question, female Fisher 344 rats received either 1) a C4 dorsal column lesion; 2) a C4 over-quadrant lesion or 3) a T3 complete transection SCI followed by NPC grafts and serial assessment of sensory outcomes. NPC grafts were obtained from GFP-expressing rat embryonic day 14 spinal cords, and were implanted into lesion sites with growth factor cocktail 1 or 2 weeks after SCI. Forelimb sensory outcomes were evaluated up to 3 months post-grafting. Neither C4 dorsal column lesion nor over-quadrant lesion (without graft) led to alterations in forelimb sensory outcomes, and implantation of NPC graft had no impact on either tactile or thermal withdrawal thresholds; thus, NPC grafts directly into spinal cord segments innervating the forepaws did not *cause* neuropathic pain.
T3 complete transection lesions resulted in the development of forelimb neuropathic pain behaviors, including forepaw spontaneous lifting, tactile allodynia and cold sensitivity, as we previously described (Lee-Kubli et al., 2016). Implantation of NPC grafts into the T3 lesion had

no effect on the development and maintenance of these neuropathic pain behaviors. The impact of NPC grafts on previously identified markers of SCI-induced neuropathic pain (Iba1, GFAP and CGRP) were also evaluated. Notably, rats that received an NPC graft showed significantly increased CGRP fiber sprouting in the cervical spinal cord segments, but did not impact the extent or magnitude of lesion-induced sensory function.
A consideration of great importance in this work is whether implants of NPC grafts can modify, either beneficially or detrimentally, SCI-related pain outcomes. These findings indicate that NPC grafts after SCI do not generate or worsen neuropathic pain states, supporting the safety and feasibility of this approach for potential clinical translation.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

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NIH RO1OD018272

Title: Long-term survival of spinally grafted porcine syngeneic or allogeneic iPS-derived neural precursors in naive or chronic spinally-injured pigs.

Authors: ***S. MARSALA**[1], J. STRNADEL[1], C. CARROMEU[2], O. PLATOSHYN[1], M. R. NAVARRO[1], S. JUHAS[5], J. JUHASOVA[5], K. YAMADA[6], T. KATO[7], J. BUI[3], E. I. CURTIS[4], J. D. CIACCI[4], M. MARSALA[1];
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Abstract: Background: The use of induced pluripotent stem cell-derived neural precursor cells (iPS-NPCs) represents a promising strategy for generation of cells with the ability to repopulate and repair the injured spinal cord. The possibility of using autologous or syngeneic cells for tailored therapy with no need for immunosuppression would represent a significant improvement over currently used allogeneic cell replacement protocols which require immunosuppression. In

our current study, we have tested the engraftment of porcine iPS-NPCs after transplantation into the lumbar spinal cord of syngeneic non-injured pigs or in allogeneic transiently-immunosuppressed recipients with spinal (L3) injury. Material and Methods: Porcine iPS were derived from skin fibroblasts of MHC-inbred pigs and reprogrammed with Sendai virus. NPCs were isolated by harvesting of the group of cells (5-10 cells), with star-like morphology and emerging from induced neural rosettes. NPCs were further expanded, characterized in vitro by calcium imaging and immunofluorescence staining. Clones of SYN-GFP expressing NPCs were then prepared and used in vivo for grafting into the: i) striata of immunodeficient rats, ii) lumbar spinal cord in syngeneic non-injured pigs without immunosuppression, and iii) lumbar spinal cord in allogeneic transiently-immunosuppressed pigs with previous spinal cord injury. After cell grafting, animals survived for 1-6 months and the presence of grafted cells confirmed with immunofluorescence staining. Results: i) In vitro induced NPCs showed expression of neural/neuronal markers, including DCX, NeuN, GABA and GFAP. ii) After in vivo grafting into the striata of immunodeficient rats, the NPCs showed a robust engraftment and expression of mature neuronal and non-neuronal markers (NeuN, NSE, SYN, GFAP). A preferential GABA-ergic phenotype was seen in grafted neurons. Extensive axonal projection into the host tissue and development of putative synapses with neurons of the host was also seen. iii) Analysis of grafted NPCs in syngeneic recipients showed similar long-term survival and neuronal/glial differentiation. No graft rejection or tumor formation was noted. iv) NPCs grafted into allogenic recipients with previous spinal injury showed comparable robust survival and incorporation into the host-previously injured spinal cord tissue. No signs of graft rejection were seen 3 months after immunosuppression was terminated. Conclusion: These data demonstrate that the use of syngeneic or allogeneic iPS-derived NPCs can successfully be used in large animal models for testing the long-term efficacy of cell-replacement therapy in spinally injured animals.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Title: Transplantation of human embryonic stem cell-derived immature astrocytes promotes long-term survival and functional recovery after spinal cord injury

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Abstract: Contusive spinal cord injuries (SCI) often lead to severe and persistent impairments of sensorimotor functions and are clinically the most frequent type of SCI. Transplantation of human embryonic stem cell (hESC)-derived immature astrocytes could be an attractive strategy to improve anatomical reorganization and functional recovery after SCI. However, such an innovative strategy remains to be tested in animal models of SCI. In the present study, we investigated the therapeutic potential of intraspinal transplantation of hESC-derived immature astrocytes into immune deficient rats following a moderate spinal cord contusive injury at the 10^{th} (T10) thoracic vertebral level using a NYU/MASCIS impactor (10 gm dropped from a height of 12.5 mm). Ten days after the injury, hESC-derived immature astrocytes or vehicle medium were injected into the lesion center as well as rostral and caudal to it. Animals received routine behavioral, electrophysiological, and urinary bladder assessments up to 9 months post-transplantation. We demonstrated that transplanted hESC-derived immature astrocytes survived well within the injured host spinal cord for a considerable period of time (up to 9 months), migrated extensively for a long distance, differentiated into mature astrocytes, and integrated into the injured host spinal cord. In addition, transplantation of hESC-derived immature astrocytes enhanced myelin formation and tissue sparing, and decreased the inhibitory molecules associated with glial scar formation as compared to vehicle-treated control animals. Moreover, >80% of rats receiving hESCs-derived immature astrocytes recovered transcranial magnetic motor-evoked potential (tcMMEP) responses, indicating that conduction through axons across the lesion was partially restored. Lastly, transplantation of hESC-derived immature astrocytes promoted long-term recovery of both locomotor and urinary bladder functions. Importantly, no tumor formation was observed in rats receiving hESC-derived immature astrocyte transplantation. Collectively, these findings suggest that hESC-derived immature astrocytes may be a unique cell type for transplantation-mediated reorganization and recovery of function following SCI.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Wings for Life

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Title: A combinatorial treatment strategy following spinal cord injury: Human stem cell-derived neuronal grafts and local delivery of chondroitinase ABC

Authors: *T. FUEHRMANN[1], P. ANADAKUMARAN[1], S. L. PAYNE[1], M. PAKULSKA[1], B. VARGA[2], A. NAGY[2], C. TATOR[3], M. S. SHOICHET[1];
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Abstract: Spinal cord injury (SCI) is a devastating condition leading to loss of sensory and motor function. The initial mechanical injury and subsequent secondary events result in substantial cell loss, and in the formation of an inhibitory environment that prevents endogenous repair. Cell transplantation to replace lost cells is a promising strategy to promote tissue repair and functional recovery following SCI; however, cell survival and integration into host tissue is limited. Although some interventions have shown promising results, it is unlikely that any one strategy, alone, would be able to promote dramatic functional improvements. It is more likely that combined approaches will yield greater benefits. This project focuses on combining the pro-survival effect of an injectable hydrogel on pre-differentiated human induced pluripotent stem cell-derived neuroepithelial cells (hNECs) combined with the local delivery of chondroitinase ABC (ChABC).Previous research indicates that pre-differentiation towards a neuronal lineage enhances cell survival. As a result, hNECs were differentiated into immature neurons, as indicated by the down-regulation of neural stem cell marker (SOX2, Nestin) and the up-regulation of neuronal marker (beta-III-tubulin, doublecortin). Cells were sorted for PSA-NCAM prior to grafting. Additionally, it has been shown that the physical blend of hyaluronan (HA) and methylcellulose (MC) promotes cell survival after grafting. HA is shear thinning and can be delivered through a fine needle, whereas MC is inverse thermal gelling, enabling it to form a gel at 37°C to provide localized delivery. One week following a moderate clip compression injury

(26g) at level T2 animals (female rats) received cell transplants in HAMC (0.75% / 0.75%) at 4 sites around the lesion (160.000 cells in total). Animals were tested for motor (BBB, ladder walk) and sensory function (tail flick) for up to 9 weeks.To promote integration of the neuronal cells, we co-delivered ChABC into the intrathecal space using a crosslinked MC hydrogel modified with the SRC Homology 3 domain for affinity release. ChABC degrades chondroitin sulphate proteoglycans (CSPGs), which are part of the inhibitory environment after injury. It can also degrade perineuronal nets, potentially promoting synapse formation between grafted and endogenous neurons.The combined therapy did not have any deleterious effects on motor or sensory function, demonstrating that the neuronal cells, ChABC and delivery vehicles are safe. Early survival of the hNECs-derived neurons was observed. We are currently evaluating the long term survival of grafted cells and the effect of ChABC on cell integration.

Disclosures: T. Fuehrmann: None. **P. Anadakumaran:** None. **S.L. Payne:** None. **M. Pakulska:** None. **B. Varga:** None. **A. Nagy:** None. **C. Tator:** None. **M.S. Shoichet:** None.

Poster

522. Regenerative Approaches: Spinal Cord Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 522.16/NN4

Topic: C.09. Brain Injury and Trauma

Support: CIHR Grant MOP 133721

Manitoba Paraplegic Foundation

Title: Development and characterisation of PLGA biodegradable nanocarriers for controlled and sustained delivery of Neuregulin-1 in spinal cord injury

Authors: K. SANTHOSH[1], A. ALIZADEH[1], *S. KARIMI-ABDOLREZAEE[2];
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Abstract: Spinal cord injury (SCI) leads to significant cell death and tissue degeneration that underlies functional impairments. Pharmacological interventions to promote cell replacement holds great promise for SCI repair. We previously showed the potential of Neuregulin-1 (Nrg1) therapy in enhancing oligodendrocyte differentiation after SCI. Nrg1 is a key growth factor for proliferation and oligodendrocyte differentiation of neural precursor cells (NPCs). Here, we developed an injectable poly (lactic-co-glycolic acid) (PLGA) nanocarrier based system for Nrg1 delivery into the injured spinal cord. Nrg1 peptide was encapsulated into PLGA nanocarriers by water-oil-water double emulsion method. Porosity was introduced by incorporating sodium

bicarbonate and later salt leaching. We assessed the release rate of Nrg1 from PLGA nanocarriers with different degree of porosity. Porosity was directly proportional to the release rate of Nrg1 and inversely proportional to loading efficiency. Distribution analysis showed average particle size was directly proportional to duration of Nrg1 release. Assessment of various PLGA-Nrg1 nanocarrier preparations identified that 1.0 mg $NaHCO_3$ as porogen and 3 min sonication results in a release rate of 255.1±0.9ng/ml and prolonged release of 21 days for preparation with mean particle size of 1.42 μm and for 42 days delivery for mean particle size of 2.37 μm. Using complementary *in vitro* and *in vivo* approaches, we tested the safety and efficacy of PLGA nanocarriers and Nrg1 release. In primary cultures of adult NPCs, the PLGA was found to be non-toxic with no detrimental effects on NPC proliferation and differentiation. Moreover, in primary cultures of mixed astrocytes/microglia, PLGA did not elicit glial reactivity. Interestingly, PLGA-Nrg1 or Nrg1 alone similarly promoted NPCs proliferation and their differentiation into oligodendrocytes suggesting the efficacy of PLGA in releasing bioactive Nrg1. In a clinically relevant model of compressive SCI in rats, intraspinal PLGA-Nrg1 injection maintained significantly higher tissue levels of Nrg1 compared to Nrg1 delivered intrathecally by mini osmotic pumps. Electron-micrographs confirmed the presence of PLGA particles inside the spinal cord with a size range of 0.2-1.87 μm. Our time-point tissue assessments also showed beneficial effects of PLGA-Nrg1 therapy in attenuating scar formation after SCI. In conclusion, our study shows that PLGA nanocarriers can be used for local and sustained delivery of bioactive Nrg1 within the SCI tissue. This system allows simultaneous delivery of multiple agents in pharmacological and cellular therapies for the treatment of SCI.

Disclosures: K. Santhosh: None. **A. Alizadeh:** None. **S. Karimi-Abdolrezaee:** None.

Poster

522. Regenerative Approaches: Spinal Cord Injury

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Program#/Poster#: 522.17/NN5

Topic: C.09. Brain Injury and Trauma

Support: The Miami Project to Cure Paralysis

Craig H. Neilsen grant # 284621

Title: The effect of mesenchymal stem cells combined with an angiopoietin-1 mimetic on blood spinal cord barrier stabilization after spinal cord injury

Authors: *M. OUDEGA[1], M. M. MARLOW[2], G. J. RITFELD[2];
[1]Dept. of Neurolog. Surgery, The Miami Project to Cure Paralysis, Univ. of Miami Dept. of Neurolog. Surgery, Miami, FL; [2]The Miami Project to Cure Paralysis, Miami, FL

Abstract: Bone marrow-derived mesenchymal stem cell (MSC) transplantation is a promising strategy for repair of the injured spinal cord. MSCs elicit neuroprotection associated with partial functional recovery. A possible mechanism underlying MSC-mediated neuroprotection is the promotion of an angiogenic response by secreted angiogenic growth factors, including vascular endothelial growth factor (VEGF). However, newly formed blood vessels may contribute to unwanted inflammatory processes due to an immature blood-spinal cord-barrier (BSCB) that allows leakage. In the present study, we combined MSC transplantation with a mimetic of angiopoietin-1 to accelerate the maturation of the BSCB of both MSC-induced new blood vessels and endogenous blood vessels. In adult female Sprague-Dawley rats we elicited a moderately severe T9 spinal cord contusion. Three days later, we injected 5 x 10^5 MSCs in the contusion epicenter and started a daily systemic treatment for 7 days with angiopoietin-1 mimetic. Spinal cords were collected at 8 days, 17 days, and 4 weeks post contusion. The BSCB was analyzed structurally with antibodies against Occludin and SMI-71 and functionally by antibodies against fibrinogen and albumin. Maturation of the BSCB was correlated with spared tissue volumes, inflammatory markers, and functional recovery. Our data provides detailed information about the feasibility of combining an angiogenic strategy (MSC transplantation) with a BSCB maturation enhancing therapeutic (angiopoietin-1 mimetic) for tissue repair after spinal cord injury.

Disclosures: **M. Oudega:** None. **M.M. Marlow:** None. **G.J. Ritfeld:** None.

Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: C.09. Brain Injury and Trauma

Support: Veterans Adminstration

NIH

the California Institute for Regenerative Medicine

the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Prolonged human neural stem cell maturation in the injured central nervous system

Authors: *P. P. LU[1,2], S. CETO[1], L. GRAHAM[1], H. KUMAMARU[1], E. BOEHLE[1], M. H. TUSZYNSKI[1,2];
[1]UCSD, La Jolla, CA; [2]VA-San Diego Healthcare Syst., San Diego, CA

Abstract: Human embryonic stem cell (H9)-derived neural stem cells (NSCs) were implanted into sites of spinal cord injury in immunodeficient rats and assessed over 18 months. Notably, grafts evidenced continued maturation over this entire time period. Immature neurons labeled with doublecortin appeared as early as 1 month post-grafting, but mature neuronal markers, NeuN, were first expressed three months post-grafting. To assess the total numbers of grafted human cells expressing a neuronal marker at all time points, we selected Hu as an immature and mature neuronal marker. Hu-labeled neurons continued to progressively enlarge in size while total numbers declined at early times and then partially recovered, indicating dynamic neurogenesis. While axons emerged early from grafts in very high numbers and extended over very long distances, only half of these projections persisted by 18 months. Mature astrocytic markers first appeared only after 6 months, while mature oligodendrocyte markers were not present until 12 months post-grafting. Astrocytes slowly migrated out from the graft over time. Graft size was stable over time and few dividing cells remained after 18 months. Thus, human NSCs retain an intrinsic *human* rate of maturation despite placement in the traumatic rodent environment, a finding of great importance in planning and assessing human clinical trials.

Disclosures: P.P. Lu: None. **S. Ceto:** None. **L. Graham:** None. **H. Kumamaru:** None. **E. Boehle:** None. **M.H. Tuszynski:** None.

Poster

522. Regenerative Approaches: Spinal Cord Injury

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Program#/Poster#: 522.19/NN7

Topic: A.04. Transplantation and Regeneration

Support: Brown Institute for Brain Science Graduate Research Award

NASA Rhode Island Space Grant Graduate Fellowship

Title: Microtissue-derived matrices reveal specific neural niche architectures

Authors: *E. B. EVANS, M. LUMINAIS, D. HOFFMAN-KIM;
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Abstract: Background: Several neurological pathologies may benefit from cell transplant therapy; however, enhanced CNS regeneration from successful cell therapy relies on the ability

of the transplanted cells to adhere, survive, and migrate into the targeted lesion site. Given the low viability and poor positional targeting of existing cell transplant methods, there is a need to develop biomaterials, which when co-delivered with grafted cells, improve cell survival and retention at the delivery site. Towards this end, one regenerative biomaterial strategy is to decellularize donor tissues to generate biochemical and physical cues from healthy extracellular matrix (ECM). Although decellularization methods have recently been reported for whole brains, it is unknown whether these methods can be applied to engineered tissues to create neural cell-derived matrices. Here, our goal was to generate three-dimensional cell-derived matrices from microtissues, to recapitulate tailorable ECM architectures.
Methods: Three different types of spheroid microtissues were generated through a self-assembly method using the following cell sources: primary rat cortical cells enriched for neurons, primary rat cortical cells enriched for astrocytes, and Neu7 cells, an inhibitory astrocyte cell line originally derived from rat cortex. We evaluated three existing decellularization protocols, including two developed for brain tissue and one previously used with tissue-engineered constructs, on 6-week old microtissues. To assess the efficacy of each decellularization method in removing cellular and nucleic material from the tissues, cryosections of microtissues were stained with Phalloidin and 4',6-diamidino-2-phenylindole (DAPI), immunostained for laminin, and processed for scanning electron microscopy.
Results: All decellularization protocols evaluated were effective at removing cellular debris from the neural microtissues, however the protocol previously used for tissue-engineered constructs best preserved the structural integrity of the ECM. Within each microtissue type, the patterning of laminin revealed distinct architectures, varying in topography and porosity.
Conclusions: Here, we show that acellular matrices can be derived from engineered neural microtissues. As a proof of concept, we have demonstrated through the culture and subsequent decellularization of three different example microtissues, representative of various cerebral cortex niches, that matrix architecture can be engineered through the inclusion of specific neural cell populations.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

Location: Halls B-H

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Program#/Poster#: 522.20/NN8

Topic: E.09. Spinal Cord Injury and Plasticity

Title: Cervical spinal cord injury degenerates distal locomotor central pattern generator (CPG)

Authors: *K. SATKUNENDRARAJAH[1], S. K. KARADIMAS[2], S. GOSGNACH[3], M. G. FEHLINGS[2];
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Abstract: Spinal cord injury (SCI) is devastating, causing significant locomotor deficits and potential paralysis with no optimal treatment available to date. Thus far, barring SCI directly to the segments housing the locomotor CPG, it is presumed that these networks remain intact after trauma. Stemming from this optimistic presumption, many approaches for restoring motor function below the injury site have focused on reestablishing the necessary excitation by either coaxing axons across the lesion to their synaptic partners, or directly activating the CPG using electrical stimulation. However, the extent to which intrinsic anatomical properties of these distal circuits remain intact following rostral SCI has been conspicuously overlooked. Using mice and human with progressive cervical SCI (cSCI), first we demonstrate significant locomotor deficits such as reduced speed and cadence as well increased stance phase duration compared to uninjured controls. In addition disrupted hindlimb flexor/extensor coordination and decreased ability to initiate and maintain locomotion. These locomotor disruptions were associated with significant loss of neurons (NeuN+) within the ventromedial regions of the distal lumbar spinal cord in cSCI animals. Interestingly, this neuronal loss was also mirrored in human cSCI patients. To further examine this neuronal loss, we initially induced progressive and chronic cSCI in *ChAT-GFP* mice. We found a significant loss of GFP+ cells within lamina IX of the lumbar enlargement in cSCI compared to sham ChAT-GFP mice. Moreover, preserved motoneurons displayed altered morphological characteristics such as decreased dendritic arborizations and decreased soma size. Glutamatergic neurons in the ventromedial area of the lumbar spinal cord are critical for rhythm generation and for initiating and maintaining locomotor activity. Hence, we specifically assessed the integrity of glutamatergic neurons by inducing progressive cSCI in double transgenic *Vglut2::cre; tdtomato* mice. Unbiased stereology and 3D reconstruction demonstrate significant loss of tdtomato cells in the ventromedial area of lumbar enlargement in cSCI *Vglut2::cre; tdtomato* compared to sham. While, we did not find differences in the number of inhibitory cells between cSCI and sham GABA(c-aminobutyric acid)/glycinergic ($VGAT^{ON}$) mice. For the first time, we present data showing that progressive and chronic cSCI induces specific anatomical and physiological modifications of the distal locomotor CPG in the lumbar spinal cord. As such, findings of this study dramatically alter the way in which we approach the development of treatments to restore walking in cSCI patients.

Disclosures: K. Satkunendrarajah: None. **S.K. Karadimas:** None. **S. Gosgnach:** None. **M.G. Fehlings:** None.

Poster

522. Regenerative Approaches: Spinal Cord Injury

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Program#/Poster#: 522.21/NN9

Topic: E.09. Spinal Cord Injury and Plasticity

Support: CIHR MOP44358

Title: Changes in the transmission of spinal lumbar pathways following a contusion (T10) during fictive locomotion in the decerebrate/spinal cat.

Authors: *J.-P. GOSSARD, H. DELIVET-MONGRAIN, M. DEA, L. AHMED, S. ROSSIGNOL;
Dept. de Neurosciences, Univ. de Montreal, Montreal, QC, Canada

Abstract: In the field of neurotrauma, contusion models are collectively accepted to be clinically relevant. In this project, adult cats were submitted to a severe spinal contusion at T10-11 using an Infinite Horizon impactor allowing the application of force of up to 800kdynes (8N) for 30s using a flat tip (5mm). Thereafter, cats were trained to walk quadrupedally on a treadmill 5 times a week on the treadmill for 5 weeks. The recovery process was studied with EMG recordings and kinematic analyses (see poster of Delivet-Mongrain et al). Trained cats could walk unaided on all four limbs. Histological examination revealed the contusions were generally very large affecting all quadrants bilaterally with large cavities leaving almost no residual grey matter.
Contused cats were used for an acute experiment to evaluate plastic changes occurring in spinal pathways below the contusion. Two were used after 5 weeks of training. Four contused cats were further spinalized at T13: 2 recovered for 2 days and 2 for 14 days before the acute experiment. The effects of stimulating supraspinal and/or cutaneous pathways were recorded in selected nerves in both hindlimbs during fictive locomotion after decerebration and curarization.
In the 1st contused cat, we found a coordinated pattern of fictive locomotion in both hindlimbs following decerebration. In the 2nd contused cat, perineal stimulation induced locomotor rhythm in both hindlimbs but bursts were not alternating properly between left and right. Trains of stimuli in the corticospinal tracts in the right pyramids evoked an increased amplitude of locomotor bursts in ankle flexors bilaterally in the 1st cat but impeded the rhythm on the right side in the 2nd cat. Stimulation of left pyramidal tracts impeded strongly the rhythm in both cats. The amplitude of cutaneous reflexes in flexors was increased on the right side as compared to the left in the 1st cat and the opposite in the 2nd. The results indicate clearly that the integrity of the corticospinal tracts was not the same in the 2 contused cats and that cutaneous transmission was also modified differently. Our recent work showed that cats with no previous lesions display left-right symmetry in all of these aspects. In forthcoming experiments using cats with an additional spinalization, locomotor rhythm is induced by clonidine i.v. We expect to see a more coordinated

and robust locomotor pattern and a decreased cutaneous transmission after 2 weeks as compared to 2 days.
Overall the results indicate that plastic changes occurred below the contusion in spinal pathways controlling and generating the locomotor rhythm in the absence of entraining sensory feedback from the moving limbs on the treadmill.

Disclosures: J. Gossard: None. **H. Delivet-Mongrain:** None. **M. Dea:** None. **L. Ahmed:** None. **S. Rossignol:** None.

Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH grant NS048425

NIH grant NS091031

Title: The rat somatosensory corticospinal tract response to spinal injury is comparable to monkeys

Authors: M. M. MCCANN[1], A. LILAK[1], *K. M. FISHER[1], K.-A. IRVINE[2], C. DARIAN-SMITH[1];
[1]Stanford Univ., Stanford, CA; [2]Palo Alto VA Hosp., Palo Alto, CA

Abstract: We have previously shown in monkeys that different lesions of the primary afferent pathway induce very different responses in the motor and somatosensory (S1) corticospinal tract (CST) axons within the cervical spinal cord. Following a cervical dorsal root lesion (DRL), the motor CST remains robust, while the S1 CST retracts by 40% within the dorsal horn. In contrast, when a DRL is combined with a cervical dorsal column lesion (DCL; at C5), both the motor and S1 CSTs sprout dramatically into parts of the spinal grey outside the normal terminal territory. In this study, we asked if these same responses occur in the rat S1 CST following similar cervical DRL and DRL/DCL lesions. Our goal was to determine the suitability of the rat model in studying involvement of the S1 CST in post spinal cord injury recovery.
Rats were divided in to 3 groups (n=5 in each), and all animals underwent an initial laminectomy. Group 1 were controls, Group 2 were given a cervical dorsal rhizotomy at C6-7, and Group 3 received a combined DRL/DCL (where the DCL was made at the border of C5 and C6). Rats were recovered and survived for 6-8 weeks, at which time they were anesthetized, and

their S1 cortex mapped electrophysiologically to locate the region of paw representation contralateral to the lesion. This was the area most affected by the lesion and the region known to reorganize following a DRL or DRL/DCL. Anterograde tracers were injected into the area of digit representation (Lucifer yellow dextran and/or biotin dextran amine), and animals were euthanized 3 weeks later. CST axon terminal territories were then mapped in a series of coronal sections through the cervical and thoracic spinal segments using a Neurolucida system (MBF Bioscience). Results indicate that the S1 CST in the rat also retracts following a DRL but sprouts well beyond normal range when a central dorsal column lesion is included. This is similar to our observations made in the macaque and suggests a cross species response and sprouting of the S1 CST, in addition to the better known motor CST response, when a central spinal injury occurs. We will discuss the details of these similarities, and their potential role in behavioural recovery of paw function.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH DA031197

NIDA Drug Supply Program

Title: Using flow cytometry to characterize morphine-induced changes in immune function following spinal cord injury

Authors: *M. ACEVES[1,2], A. OKOREEH[1], M. N. TERMINEL[1,2], A. R. ACEVES[1], M. A. HOOK[1,2];
[1]Neurosci. and Exptl. Therapeut., Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX; [2]Texas A&M Inst. for Neurosci., College Station, TX

Abstract: Opioids are one of few effective analgesics for the treatment of pain following spinal cord injury (SCI). Unfortunately, we have shown that morphine administered in the acute phase of SCI undermines locomotor recovery, increases mortality and pain reactivity, and decreases signs of general health in a rodent contusion model. Our studies suggest that morphine produces these adverse effects by exacerbating the inflammatory response innate to SCI. Indeed, a single

administration of morphine significantly increases IL-1β at the lesion site at 30 min and 24 h post-treatment. Pre-treatment with minocycline also blocks the adverse effects of morphine on locomotor recovery. In the current study, we used flow cytometry to characterize morphine-induced changes in resident and infiltrating immune cell populations. Sham and contused (T12) subjects were implanted with an intrathecal cannula. Twenty-four hours following surgery, subjects were treated with morphine or vehicle. At 24 hours post-treatment (48 h post-surgery), 1 cm of spinal tissue encompassing the injury was collected and dissociated. The resulting cell suspension was plated and stained with markers for CD45, CD11b, IBA1, CD86, and CD68. Cells were quantified using a FACSFortessa flow cytometer, and data were analyzed using FlowJo software. We found significant main effects of surgery and drug treatment on total immune cells present at the injury site, with contused-morphine subjects showing significantly higher numbers than their contused-vehicle counterparts. Importantly, we also found a significant main effect of morphine on total and M1-polarized microglia. Irrespective of surgery, morphine increased the number of inflammatory microglia present at the injury site. Our results support the hypothesis that opioid-immune interactions underlie the adverse effects on recovery observed in our model. Morphine may bind to opioid receptors on microglia, synergistically increasing the release of pro-inflammatory factors after SCI, and producing neurotoxicity.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Rick Hansen Foundation through the ICORD-Rick Hansen Institute – Blusson Integrated Cure Partnership

Title: Testing the robustness of "promising" neuro-protective drug candidates in a cervical hemi-contusion model of rats

Authors: *W. T. PLUNET[1], N. JANZEN[2], J. LIU[2], A. BEHRENS[2], E. RAFFAELE[2], Y. JIANG[2], J. CHEUNG[2], W. WANG[2], H. JIANG[2], B. LASHKARI[2], P. ASSINCK[2], L. RAMER[2], L. MCPHAIL[2], W. TETZLAFF[2];
[1]ICORD, Univ. of British Columbia, Vancouver, BC, Canada; [2]U.B.C., Vancouver, BC, Canada

Abstract: A significant number of FDA approved drugs have demonstrated efficacy in preclinical spinal cord injury (SCI). These studies used thoracic models of contusion, yet less than 5% of human SCI are incomplete thoracic injuries. Most human injuries occur at cervical levels (>65%), half of these are incomplete and this group should benefit the most from neuroprotective treatments. The short windows of intervention used in animal studies (often less than one hour) are difficult to translate in human trials. In addition, many preclinical studies are underpowered, subject to experimenter biases or conflict of interest, which significantly reduces their value as predictors of success in a human trial. We therefore created a team of research staff to assess the effects on functional recovery of the most promising FDA approved drugs when these are administered 3 hours after a cervical spinal cord hemicontusion injury using group sizes of n=14-20.

In experiment 1 we tested riluzole (n =20) administered 3 hours after injury (control = 21) in adult male Sprague Dawley rats. RT-PCR analysis performed at 3 days after injury showed significant reductions in inflammatory markers (CD68 and CD45). In the a priori primary outcome of a skilled forelimb task (Montoya staircase: retrieval of food pellets from a staircase) there were no differences between the riluzole treated group and the control group at any of the post injury time periods (2,4,6 and 8 weeks post injury). In addition, there were no differences in the secondary outcome of proximal limb use as measured by the cylinder rearing task at 3, 5 or 7 weeks after injury.

In experiment 2 metformin (n = 19) and fluoxetine (n = 20) were tested against vehicle controls: (n = 19). In the primary outcome of skilled forelimb use, as assessed by the fruit-loop eating score on the injured side, there were no differences among the groups at 6 weeks post injury. Similarly, in the secondary behavioral outcome of proximal limb use we found no differences in the cylinder rearing task at 2, 4 or 6 weeks post injury.

In experiment 3 we examined rosuvastatin (n = 14) and inosine (n = 17) started 3 hours after injury (control = 17). In the primary outcome of distal forelimb use there were no difference in the Montoya staircase task. Similarly, in the cylinder rearing task we did not observe any differences among the groups at 2, 4, 6, or 8 weeks after injury.

As in previous replication studies, establishing robustness in preclinical models is challenging and possible reasons will be discussed.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Mission Connect Grant DA031197

Gillson Longenbaugh Foundation

Title: Antidepressant efficacy of S-ketamine in spinal cord injury

Authors: *K. BRAKEL[1,2], A. R. ACEVES[1], N. PANCHANI[1], K. NGUYEN[1], M. ACEVES[1,2], M. A. HOOK[1,2];
[1]Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX; [2]Texas A&M Inst. for Neurosci., College Station, TX

Abstract: Major depressive disorder (MDD) is a significant, but understudied, consequence of spinal cord injury (SCI). Approximately 11-24% of SCI patients experience MDD, compared to 8% in the general population. Unfortunately, however, some of the most effective antidepressants (selective serotonin reuptake inhibitors) have been associated with spasticity and poorer rehabilitation outcomes after SCI. Alternative antidepressant therapies that improve psychological well-being without compromising recovery are needed for the SCI population. To address this, we tested the efficacy of S-ketamine, an NMDA receptor antagonist that has been increasingly used as a short-term antidepressant in the clinical setting. Adult, male Sprague Dawley rats received moderate spinal cord contusion injuries. Twenty-four hours after injury, the subjects were treated with one of 4 doses of S-ketamine: 0, 5, 10, or 20 mg/kg, i.p. Depression-like behavior was assessed with a battery of established tests prior to injury and then on days 2, 9-10, and 19-21 post-injury. Locomotor function was also recorded throughout the 3-week recovery period using the Basso, Beattie, and Bresnahan (BBB) scale. To identify depression, change from baseline (collected two days before injury) scores were calculated for each post-injury test period. The change from baseline scores derived for test days 9-10 and 19-21, after any antidepressant effects of ketamine should have dissipated, were subjected to principal components and hierarchical cluster analyses. These analyses clustered the subjects into two groups: depressed and not-depressed. Thirteen of 32 subjects (41%) displayed depression-like behaviors (decreased sucrose preference and open-field activity). The incidence of depression was similar across S-ketamine dose groups. S-ketamine did not affect the long-term development of depression. However, high doses of S-ketamine (20 mg/kg) had an antidepressant-like effect at day 2, when ketamine should have been biologically active. Specifically, 20 mg/kg of S-ketamine increased social interaction in subjects later characterized as depressed. Importantly, ketamine did not compromise motor function; BBB scores were similar across all groups. While

the molecular mechanisms underlying these effects must be derived, the current study suggests that ketamine may provide a safer alternative for treating depression after SCI than traditional SSRI treatments.

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Poster

522. Regenerative Approaches: Spinal Cord Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 522.26/NN14

Topic: A.04. Transplantation and Regeneration

Support: ALS Association

Travis Roy Foundation

Massachusetts Department of Health-SCI cure

Title: Directed differentiation of corticofugal projection neurons from endogenous cortical progenitors

Authors: ***A. OZKAN**[1,3], H. PADMANABHAN[1], S. L. SHIPMAN[1], P. KUMAR[1], W. EBINA[2], A. N. BASAK[3], J. D. MACKLIS[1];
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Abstract: Specific classes of neurons are selectively vulnerable in distinct neurodegenerative, developmental, and acquired diseases of the CNS. In particular, for this work, corticospinal motor neurons (CSMN) degenerate in amyotrophic lateral sclerosis (ALS) and other motor neuron diseases, and loss of motor function in spinal cord injury results from damage to CSMN axons. Directed differentiation of new neurons with appropriate identity, maturity, circuit connectivity and function from endogenous local progenitors offers a potential therapeutic approach for functional repair of diseased or injured neuronal circuitry.
Recent work by our lab and others has begun to identify central elements of a combinatorial "molecular logic" of stage-, state-, and area-specific controls over development of broad classes and specific subtypes of cortical projection neurons. Here, we target endogenous cortical progenitors present in postnatal and adult brain to direct their differentiation into corticofugal (cortical output) projection neurons; CSMN belong to this class. Application of a select

combination of central and complementary transcriptional controls in cultured cortical progenitors directs acquisition of cardinal morphological, molecular, and electrophysiological features of corticofugal projection neurons. We employ synthetic modified RNA technology to enable temporal and dose control to mimic the *in vivo* expression dynamics of the relevant transcriptional regulators. Ongoing work will further assess fidelity of their differentiation, integration, and function within complex cortical circuitry in both developing and the diseased brain.

Disclosures: A. Ozkan: None. **H. Padmanabhan:** None. **S.L. Shipman:** None. **P. Kumar:** None. **W. Ebina:** None. **A.N. Basak:** None. **J.D. Macklis:** None.

Poster

522. Regenerative Approaches: Spinal Cord Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 522.27/OO1

Topic: A.04. Transplantation and Regeneration

Support: CNF-339836

Burke Foundation

Title: Ubiquitin Proteasome System alterations within dystrophic axonal endings following spinal cord injury

Authors: T. E. JOHNS[1], J. L. BROWN[1], *C. E. HILL[1,2];
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Abstract: After spinal cord injury (SCI), axons fail to regrow through the lesion site. Dystrophic axonal endings known as retraction bulbs form at the tips of arrested axons and persist chronically at the lesion margin. The dystrophic endings at the margins of the lesion site may provide a target for chronic SCI repair if the causes of their failure to grow can be identified and therapeutically manipulated. Although they were first described over 100 years ago by Ramon y Cajal, little is known about why retraction bulbs form and what causes them to persist after injury. This limits our ability to develop strategies to prevent their formation or reestablish the growth of chronically injured axons. In other neurological diseases, dystrophic axonal endings are associated with deficits in protein degradation. Protein degradation is necessary for the formation of growth cones following injury and their responsiveness to environmental cues, both of which are important to axonal growth following SCI. In the current study, we examined the

levels of components of the Ubiquitin Proteasome System (UPS), one of the major protein degradation systems in cells, to determine if this system is defective within dystrophic endings. Using immunohistochemistry, UPS levels were examined *in vivo* following rat SCI and *in vitro* using a proteoglycan spot assay for dystrophic ending formation. Elevated levels of the three UPS components examined (free ubiquitin, ubiquitinated protein, and proteasome core protein) were detected within dystrophic endings relative to growth cones. These results indicate that protein degradation may be altered within dystrophic endings. Manipulation of UPS activity in dystrophic endings is a necessary next step in determining whether manipulating protein degradation can overcome dystrophic axonal ending formation and promote the growth of chronically injured axons following spinal cord injury.

Disclosures: **T.E. Johns:** None. **J.L. Brown:** None. **C.E. Hill:** None.

Poster

522. Regenerative Approaches: Spinal Cord Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: A.04. Transplantation and Regeneration

Support: NIH R21NS093278

Bryon Riesch Paralysis Foundation

Title: Development of a CRISPR-based strategy for high content screening in primary neurons

Authors: *B. CALLIF, N. KRUEGER, M. T. SIMPSON, M. G. BLACKMORE;
Biomed. Sci., Marquette Univ., Milwaukee, WI

Abstract: Axon growth and regeneration are coordinated by multiple interacting proteins, the identity of which remain incompletely characterized. High content screening (HCS) combines manipulation of candidate genes with rapid image analysis of morphological changes, and has emerged as a powerful technique to identify key proteins that control axon outgrowth. Our previous screens have involved forced overexpression of candidate genes, but a complementary knockout approach is needed to clarify the role of endogenously expressed proteins. A genome editing approach often referred to as CRISPR (Clustered Regularly Interspersed Palindromic Repeats) is a rapidly developing tool for protein knockout. In the CRISPR approach, a DNA-cleaving enzyme called Cas9 is guided to target sequences in the genome by user-created guide RNA (sgRNA), where it causes a double-stranded break that ultimately results in missense mutations. Using cultured postnatal cortical neurons, we verified the ability of CRISPR to

achieve protein-level knockdown using electroporation of plasmid DNA that co-expresses Cas9 enzyme and sgRNA. Targeted proteins included NeuN, not anticipated to affect neurite length, and PTEN, a well-studied regulator of axon growth. Importantly, targeted proteins were eventually undetectable by immunohistochemistry in more than 80% of transfected neurons, but effective knockdown lagged at least four days behind transfection. Consistent with this, anti-PTEN sgRNA produced no changes in neurite outgrowth when assessed 3 days post-transfection. Therefore, we modified the procedure, such that neurons were maintained for a week post-transfection, replated to disentangle overlapping neurites, and assessed for growth after an additional 24 hours. In these replated neurons PTEN knockout produced robust increases in neurite length. These PTEN effects were achieved using multi-well transfection and automated phenotypic analysis, indicating PTEN's suitability as a positive control for CRISPR-based screening efforts. Accordingly, we have initiated a CRISPR-based knockdown screen of 50 transcription factors that potentially regulate axon growth. In addition, using viral delivery of anti-PTEN sgRNA to transgenic mice that express Cas9 we have confirmed a strong decrease in PTEN expression in adult cortical neurons in vivo. Overall these data establish CRISPR-based knockdown as effective in primary neurons, compatible with HCS workflows, and readily translated in vivo. Furthermore, our results suggest that CRISPR represents a powerful tool for discovering genetic regulators of axon growth.

Disclosures: B. Callif: None. **N. Krueger:** None. **M.T. Simpson:** None. **M.G. Blackmore:** None.

Poster

522. Regenerative Approaches: Spinal Cord Injury

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 522.29/OO3

Topic: B.12. Glial Mechanisms

Title: The olfactory ensheating cells and potencial neuroregeneration after injury: *In vitro* injury model

Authors: *M. A. DOMINGUEZ, III[1,2], R. GOMEZ BELLO[4], M. SANCHEZ MOLINA[5,4], L. BOTERO ESPINOSA[3], O. CHAPARRO GARZON[2];
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Abstract: *Aims:* The Spinal Cord Injury (SCI) is a condition in which disturbances establish a complex dysfunction. Currently, the treatment of SCI is based on procedures that seek to minimize the consequences, preserve the functionality and enhance the quality of life. In this approach, the use of cells of olfactory ensheathing cells (OEC´s) and soluble factors have been suggested as a intervention for spinal cord injuries, to be recognized, properties involved in the growth of axons of olfactory neurons.
Objective Characterize promoting and inhibitors factors expression of neuroregeneration in OEC´S interaction in vitro model of spinal cord injury.
Method
Isolation of the thoracic and spinal cord of Wistar rats. Section by cross cuts 3-5 mm. Cultured for 5 days. Then mechanical injury was performed. Once the injury was made a trial of variable dosing of previously cultured OEC's and comparison with a control group using conditioned media. Morphological evaluation by electron microscopy and quantification assay was performed by using nanotechnology biosensor.
Results Identifying the presence of promoting and inhibitory proteins (NT-4/5, NOGO-A, MAG) in cell culture and morphological changes in injured neurons pretreated with OEC's determines the involvement of these proteins in axonal regeneration after injury
Conclusion The OEC´s is a population that represents a future therapeutic alternative in the treatment of spinal cord injury because of its regenerative potential. The knowledge of protein interaction represents an approach to the mechanism involved in neuroregeneration process and the future of therapeutic possibilities based on cell therapy *Keywords*
Olfactory Ensheating Cells (OEC)Spinal Cord Injury (SCI)NeuroregenerationNanotechnology byosensor In vitro injury model

Disclosures: M.A. Dominguez: None. **R. Gomez Bello:** None. **M. Sanchez Molina:** None. **L. Botero Espinosa:** None. **O. Chaparro Garzon:** None.

Poster

522. Regenerative Approaches: Spinal Cord Injury

Location: Halls B-H

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Program#/Poster#: 522.30/OO4

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R21NS078580

Massachusetts Life Sciences Center

Title: A novel form of neuronal regeneration in *C. elegans* shares links with activity-dependent ectopic axon outgrowth and mammalian lesion conditioning

Authors: S. CHUNG, *C. V. GABEL;
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Abstract: During development, a neuron transitions from a state of rapid growth to a stable morphology, and neurons within the adult mammalian central nervous system lose their ability to effectively regenerate in response to injury. Here we identify a novel form of neuronal regeneration that is remarkably independent of the defined and well-conserved axon regeneration pathways involving DLK/p38 and MLK/JNK MAP kinase signaling. This regeneration in *C. elegans* has direct genetic and molecular links to a well-studied form of endogenous activity-dependent ectopic axon outgrowth in the same neuron type that commences during the final stages of development. Both neuron outgrowths are triggered by physical lesion of the sensory dendrite or by mutations disrupting sensory activity, calcium signaling, or genes that restrict outgrowth during neuronal maturation, such as NDR kinase or CaMKII. These connections suggest that ectopic outgrowth represents a powerful platform for gene discovery in neuronal regeneration. Moreover, we note numerous similarities between this *C. elegans* regeneration and lesion conditioning, a phenomenon producing robust regeneration in the mammalian central nervous system. Both regeneration types are triggered by lesion of a sensory neurite via reduction of neuronal activity and are enhanced by disrupting L-type voltage-gated calcium channels, elevating cAMP, or preconditioning. Taken as a whole our study unites disparate forms of neuronal outgrowth to uncover fresh molecular insights into activity-dependent control of the adult nervous system's intrinsic regenerative capacity.

Disclosures: S. Chung: None. **C.V. Gabel:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Program#/Poster#: 523.01/OO5

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS076589

NIH Grant R01NS090622

VA Grant I01RX000815

VA Grant I01RX001807

Craig H. Neilsen Foundation Grant 261299

Title: Relationship between clinical and physiological outcomes of quadriceps spasticity after spinal cord injury

Authors: *D. SOLAUN[1], C. A. PLUMLEE[2], M. A. PEREZ[1];
[1]Dept. of Neurolog. Surgery, The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL;
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Abstract: The Modified Ashworth Scale (MAS) is one of the most widely used clinical exams for assessing spasticity in humans with spinal cord injury (SCI). The extent to which MAS scores relates to other clinical and physiological measurements of spasticity after SCI remains poorly understood. In the present study, we examined spasticity in the quadriceps muscle of the more affected leg in individuals with chronic (13.6±12.6 years post injury) complete and incomplete SCI between C2 and T12 spinal segments by quantifying (i) MAS scores, (ii) the pendulum test, and (iii) input-output recruitment quadriceps H-reflex curves. The MAS assessment was performed by a certified SCI physician. The pendulum test was conducted with the subject in supine position while the examiner hold the subject's foot with the knee fully extended. Then, the examiner dropped the leg and the Vicon Motion Capture System was used to quantify the first angle of the backward swing, swing ratio (defined between the first and second angle of the backward swing), swing time (defined as the time of the first angle of the backward swing), and the total number of leg swing oscillations. The quadriceps H-reflex was tested by stimulating the femoral nerve at the femoral triangle at increasing stimulus intensities (10 pulses of 1 ms duration at each stimulus intensity delivered at 0.25 Hz). Electrophysiological outcomes included quadriceps H-reflex maximum (H-max), maximal motor response (M-max), and H/M ratio. In the pendulum test, we found that MAS scores were negatively correlated with the total number of swing oscillations ($p<0.001$) but not with the first angle of the backward swing and swing time and ratio ($p>0.05$). Physiological measurements showed that MAS scores showed a weaker relationship with quadriceps H-max, M-max and H/M ratio. Altogether, our findings indicate that an aspect of the pendulum test, specifically the total number of swing oscillations, is more likely to reflect changes detected by the MAS clinical exam following human SCI.

Disclosures: D. Solaun: None. **C.A. Plumlee:** None. **M.A. Perez:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS076589

NIH Grant R01NS090622

VA Grant I01RX000815

VA Grant I01RX001807

Craig H. Neilsen Foundation Grant 261299

Title: Time-dependent discrepancies between assessments of sensory function after incomplete cervical spinal cord injury

Authors: *R. A. MACKLIN[1], P. H. ELLAWAY[2], M. A. PEREZ[1];
[1]Dept. of Neurosurg., Univ. of Miami, Miami, FL; [2]Div. of Brain Sci., Imperial Col., London, United Kingdom

Abstract: We recently demonstrated that the electrical perceptual threshold (EPT) reveals spared sensory function at lower spinal segments compared with the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) exam in humans with chronic incomplete cervical spinal cord injury (SCI). Here, we investigated whether discrepancies in sensory function detected by both sensory exams change over time following SCI. Forty eight participants with acute (< 1 year), chronic (> 1 to 10 years), and extended-chronic (> 10 years) incomplete cervical SCI and 30 control subjects were tested on dermatomes C2 to T4 bilaterally. EPT values were higher in subjects with acute (2.5±0.8 mA), chronic (2.2±0.7 mA), and extended-chronic (2.8±1.1 mA) SCI compared with controls (1.0±0.1 mA). The EPT exam detected sensory impairments in spinal segments above (2.3±0.9) and below (4.2±2.6) the level detected by the ISNCSCI sensory exam in individuals with acute and chronic SCI, respectively. Notably, both exams detected similar levels of spared sensory function in the extended-chronic phase of SCI (0.8±0.5). A negative correlation was found between differences in EPT and ISNCSCI sensory levels and time post injury. These observations indicate that discrepancies between EPT and ISNCSCI sensory scores are time-dependent, with the EPT revealing impaired sensory function above, below, or at the same spinal segment as the ISNCSCI exam over time following SCI. We propose that the EPT is a sensitive tool to assess changes in sensory function throughout time after incomplete SCI.

Disclosures: R.A. Macklin: None. **P.H. Ellaway:** None. **M.A. Perez:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Program#/Poster#: 523.03/OO7

Topic: C.09. Brain Injury and Trauma

Support: NIH NINDS R01 NS081112

Title: Transplanted neural precursor cells integrate with the injured cervical spinal cord

Authors: L. V. ZHOLUDEVA, V. M. SPRUANCE, T. G. BEZDUDNAYA, K. M. NEGRON, I. FISCHER, *M. A. LANE;
Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: More than half of all spinal cord injuries (SCIs) occur at the cervical level resulting in some degree of respiratory deficiency. Although there has been increasing evidence for spontaneous neuroplasticity and improvements in respiration, functional recovery remains limited. Thus, there is an urgent need for the development of therapies targeting restoration of respiratory function following SCI. One therapeutic strategy gaining translational recognition is transplantation of neural precursor cells (NPCs) to repair the injured spinal cord. NPC transplants have been shown to survive, differentiate, form synaptic connections between donor and host cells, and enhance functional recovery. However, little is known about the development of donor neurons transplanted into the injured spinal cord, and whether the injured adult spinal cord selects for specific neuronal phenotypes that could limit therapeutic potential. The present work begins to track the development of cultured and non-cultured NPCs 3, 7, 14 and 30 days after transplantation into a lateral, C3/4 contusion injury in the adult rat. Results from these ongoing studies reveal that grafted cells survive and differentiate into mature neurons and glia within the injured spinal cord 14 days post-transplantation. However, cell survival and neurite outgrowth appears to be greater in non-cultured grafts. The neuronal precursor phenotype also differs between cultured and non-cultured donor NPCs. These ongoing experiments are beginning to identify donor cell phenotypes and offer insight into how the internal milieu of the injured spinal cord might select for specific neurons and their outgrowth into host tissue.

Disclosures: L.V. Zholudeva: None. **V.M. Spruance:** None. **T.G. Bezdudnaya:** None. **K.M. Negron:** None. **I. Fischer:** None. **M.A. Lane:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Program#/Poster#: 523.04/OO8

Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R01-NSO81112

Title: Short and long term effects of neural progenitor transplantation following cervical spinal cord contusion injury

Authors: *V. SPRUANCE[1], L. ZHOLUDEVA[2], K. NEGRON[3], T. BEZDUDNAYA[3], M. LANE[3];
[1]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Drexel Univ., Philaldelphia, PA; [3]Drexel Univ., Philadelphia, PA

Abstract: Impaired breathing is a devastating consequence of cervical spinal cord injury (SCI) that increases morbidity and the risk of mortality. Injuries at high-to-mid cervical levels (C1-4) result in the most severe deficits as the phrenic motor circuitry - controlling the diaphragm - is directly compromised, typically resulting in dependence on assisted-ventilation. While there is mounting evidence for spontaneous respiratory improvement, the extent of recovery - or functional plasticity - remains limited. Thus, there is a need to develop therapeutic strategies for enhancing repair and recovery of respiratory pathways.
Our ongoing research aims to elucidate spinal and supraspinal changes that may influence respiration post-SCI, and assess whether treatments can harness ongoing neuroplasticity to improve function post-injury. These studies have identified that spinal interneurons represent a potential therapeutic target for enhancing plasticity and recovery of phrenic motor function. With a particular focus on the phrenic motor system, the goal of the present work is to assess whether transplantation of neural precursor and stem cells (NPCs/NSCs) can facilitate repair of the injured adult rat cervical spinal cord and promote lasting, functional recovery. We hypothesize that spinally derived NPCs, rich in interneuronal precursors, will provide a source of neurons that facilitate a novel neuronal relay capable of restoring input to phrenic motoneurons.
Adult, female Sprague-Dawley rats (~250g) received lateralized C3/4 contusions (200 kilodynes, Infinite Horizons Pneumatic Impactor). One week post-injury, NPCs derived from developing rat spinal cord (E13.5 Sprague Dawley or E13.5 Fisher rat, expressing green fluorescent protein) were injected directly into the injury cavity (~1 million cells). Transplanted animals are compared against injured, untreated animals. Four weeks or one year later, a transynaptic, retrograde tracer (pseudorabies virus) was delivered to the ipsilateral hemidiaphragm or directly into the transplant. Tracing revealed synaptic integration between donor neurons and host phrenic circuitry at one month following transplantation. However, evidence for this connectivity is lost at one year following transplantation. Terminal electrophysiology analysis revealed variable phrenic and diaphragm recovery at both time points in those animals that received NPC transplants following cervical contusion injury. These ongoing studies are providing insight into the therapeutic potential for NPC therapy in the injured spinal cord.

Disclosures: V. Spruance: None. **L. Zholudeva:** None. **K. Negron:** None. **T. Bezdudnaya:** None. **M. Lane:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Program#/Poster#: 523.05/OO9

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH/NINDS Diversity Supplement Award NS056413

Christopher & Dana Reeve Foundation

Walkabout Foundation

Title: Tibialis anterior muscle activity during the paw withdrawal learning paradigm

Authors: *M. S. JOSEPH[1], K. GRIFFIS[1], H. ZHONG[1], R. R. ROY[1,2], N. J. K. TILLAKARATNE[1,2], V. EDGERTON[1,2,3];
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Abstract: The spinal cord has the ability to learn motor tasks in the absence of supraspinal input. The paw withdrawal learning (PaWL) paradigm represents a simple spinal learning model. In mice whose spinal cords are completely transected (ST) at mid-thoracic level, tibialis anterior (TA) muscle is given a shock to a hind leg when the leg is extended. The master mice learn to maintain the shocked leg in a flexed position while the experimentally coupled yoked group do not. To demonstrate the acquisition of the de novo flexed paw hold is mediated through muscle-specific proprioceptive inputs and a time dependent modification of the spinal interneuronal network associated with the TA motor pool, we measured the TA muscle activity using acute EMG during PaWL in both master and yoked mice. By blocking the proprioceptive muscle and the cutaneous afferents using Lidocaine, we demonstrated that TA- specific muscle afferents are critical in PaWL. In master TA muscle we detected greater activity when compared to the yoked during PaWL. Furthermore, the TA muscle showed significant EMG activity when compared to medial gastrocnemius and vastus lateralis muscles. During maintenance period of paw dorsiflexion within the master group, we find significant increase in paw displacement above the threshold coupled with decreasing iEMG activity suggesting the increase in network efficiency in performing the paw flexion. The significant increase in the EMG amplitude in the TA muscle and time-frequency and power relationships in the master group, but not the yoked, suggests that the successful learning requires temporally mediated engagement of the spinal sensorimotor network.

Disclosures: **M.S. Joseph:** None. **K. Griffis:** None. **H. Zhong:** None. **R.R. Roy:** None. **N.J.K. Tillakaratne:** None. **V. Edgerton:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

Location: Halls B-H

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Program#/Poster#: 523.06/DP06 (Dynamic Poster)

Topic: E.09. Spinal Cord Injury and Plasticity

Support: UCLA CTSI UL1TR000124

Bel13ve Foundation

Walkabout Foundation

Christopher and Dana Reeve Foundation

Title: Use of pact clearing technique to identify and trace activated neurons in the spinal network during locomotor behavior

Authors: *B. N. PHAM, JR, H. ZHONG, N. J. K. TILLAKARATNE, V. EDGERTON; UCLA, Los Angeles, CA

Abstract: The performance of motor tasks such as standing or stepping requires integration of cutaneous and proprioceptive inputs across many spinal segments. The role of these hindlimb inputs on locomotor recovery after spinal cord injury (SCI) has been shown behaviorally and electrophysiolgically, but little is known of the spatial distribution and type of neurons at the network level. Understanding the composition of this network and the changes it undergoes after SCI necessitates the need to analyze the spinal cord on a macro network level that is difficult to interpret using standard histological techniques. To address this difficulty we used tissue clearing techniques to gain a macro-perspective on spinal locomotor networks as they function *in vivo*. Adult C57/Bl6 mice had intramuscular injections of the transynaptic tracer pseudorabies virus (PRV) and the retrograde tracer Cholera Toxin subunit B (CTB) into the tibialis anterior (TA) and soleus (SOL), respectively. After 72 or 96 hrs post-viral injections, these mice were quadrupedally stepped for 60 minutes on a moving treadmill and terminated one hour later. The spinal cords were cleared using the Passive CLARITY Technique (PACT) protocol and subsequently underwent whole tissue immunohistochemistry (IHC) for the c-fos, CTB, and GFP antibodies. C-fos was used to demarcate activated spinal neurons. The tissue was then imaged using a Leica SP5 confocal and rendered using the Vaa3D program.
We found that the locations of Fos+ neurons in PACT-cleared tissue were similar to a previous study in which quadrupedally-stepped rat tissue was processed by standard IHC in 30 μM-thick sections (Ahn et al. 2006). The majority of Fos+ neurons were located in laminae V-VII with sparse labeling in laminae I-III and IX. PACT clearing was able to reveal strong labelling of PRV+ TA neurons, CTB+ SOL neurons, and CTB+ afferent terminals. The spread of PRV+

neurons were similar in PACT and 30 μM-thick tissue, with 72 hr infection labeling mostly motoneurons and that 96 hr infection labeling many interneurons. The localization of CTB+ terminals in lateral laminae II-IV and medial lamina V and VI was similar to that in 30 μM-thick sections (Hirakawa et al. 1992; Tillakaratne 2014). PACT clearing of spinal cord tissue allowed for faster tissue processing times over standard IHC. The combination of neural tracing, IHC, and PACT clearing enables increased three dimensional resolution that better captures complex, neural network connectivity. The application of PACT to analyze mouse spinal cords on a network level will facilitate the identification of network level changes that occur in the mouse spinal cord after SCI and subsequent rehabilitation.

Disclosures: B.N. Pham: None. **H. Zhong:** None. **N.J.K. Tillakaratne:** None. **V. Edgerton:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 523.07/OO10

Topic: E.09. Spinal Cord Injury and Plasticity

Title: Possible mechanisms underlying conversion of nonfunctional to functional states among spinal networks after spinal cord injury

Authors: *K. A. DEPETRO, S. ZDUNOWSKI, N. J. K. TILLAKARATNE, B. PHAM, H. ZHONG, V. R. EDGERTON;
UCLA, Los Angeles, CA

Abstract: Our previous studies show that epidural and transcutaneous spinal stimulation and step/stand training can restore voluntary control in adult humans with chronic, clinically motor complete spinal cord injuries. However, the mechanism by which voluntary improvements recover seems too early to be attributed to axonal growth across the lesion. We hypothesize that some spinal networks can survive in a nonfunctional state after injury, but can be converted into a functional state and form functional connections to networks below the lesion and to motor pools. Previous studies done in rats have shown that in a hemisection (HX) model, 1-2 weeks post-injury, anatomically intact spinal axons, contralateral to the lesion, show dispersal of voltage-gated sodium channel isoform 1.6 (Nav1.6) and characteristics of demyelination associated with reduced conduction. Nav1.6 is the predominant voltage-gated sodium channel at the nodes of Ranvier in the adult CNS, and is largely responsible for depolarization. The current study seeks to determine whether this mechanism can be a factor in the recovery of voluntary control. Adult female Sprague-Dawley

rats were divided into hemisected, hemisected with step/stand training, and hemisected with ES and step/stand training. Rats were: 1) pre-trained before injury, 2) injected with anterograde tracer into the hindlimb motor cortex, 3) implanted with an epidural stimulator and transcranial stimulator with electrodes placed bilaterally into the soleus (SOL) and tibialis anterior (TA), 4) hemisected, and 5) step trained and stimulated, and 6) tested for stepping ability and motor evoked potentials (MEPs) in implanted muscles. Tissue was analyzed for dispersal of Nav1.6 channels from the nodes of Ranvier and myelin was assessed. Electrophysiological analysis showed bilaterally delayed conduction through the spinal cord post-injury followed by gradual improvement in the SOL and TA, followed by a drastic decline in MEP amplitude at each stimulation intensity (4.0 mA, 2.0 mA, 1.0 mA, and 0.5 mA) at day 15. This is consistent with the previous studies showing dispersal of Nav1.6 from the nodes of Ranvier around weeks 1-2 post-HX. The effect of Nav1.6 channel dispersal with the use of epidural stimulation and training and how these variables relate to the restoration of function will be presented.

Disclosures: K.A. Depetro: None. **S. Zdunowski:** None. **N.J.K. Tillakaratne:** None. **B. Pham:** None. **H. Zhong:** None. **V.R. Edgerton:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

Location: Halls B-H

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Program#/Poster#: 523.08/OO11

Topic: C.09. Brain Injury and Trauma

Support: Veterans Affairs Rehab R&D Merit Review #1I01RX001005-01A2

Title: Simultaneous application of therapeutic exercise and magnetic stimulation significantly improved cervical spinal cord injury (C-SCI)-induced spasticity and gait parameters

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Abstract: In human, more than 50% of spinal cord injuries occur at the cervical level. Following C-SCI, spasticity and gait disabilities are two most common complications which can significantly affect quality of life. Recently, we reported that a combination of treadmill (Tm) locomotor exercise with SCI-site magnetic stimulation yielded significant locomotor improvement, and spasticity reduction that was greater than either treatment tested alone (Hou et al., 2014). In order to further improve the efficacy of the therapy, here, we evaluated the

therapeutic effects of simultaneous application of Tm locomotor training with injury-site magnetic stimulation. Moderate C6/7 contusion injuries (200 kdynes, Infinity Horizon Impactor) were produced in 20 anesthetized adult Sprague-Dawley rats. Ten rats were randomly selected and initiated Tm locomotor therapy at post-injury (p.i.) day 8. Started from p.i. week 2, magnetic stimulation was applied at the injury site using a single pulse protocol that we have recently reported (Hou et al., 2014). Magnetic stimulation was provided on every other day for 6 weeks while rats were walking on the Tm. Exercise protocol continued 5 days a week and all treatments ended at p.i. week 8. The velocity-dependent ankle torques and time-locked triceps surae EMGs were recorded at p.i. week 4 and week 8 as a measure of spasticity. Six weeks of magnetic stimulation combined with the Tm locomotor training protocol completely blocked the development of spasticity when compared to data obtained from untreated injured controls. In addition, plantar H-reflex rate-depression was also measured following completion of the treatment to index quantitative changes in inhibitory processes that regulate motoneuron excitability. The combined treatment group showed rate-depression similar to that recorded in the normal controls. Moreover, the combined treatment group showed significantly increases (68.37%) in grip strength, and significantly fewer errors in paw/foot placement on a stepladder compared to corresponding data obtained from the untreated injured controls. Combined treated animals also exhibited an improved and normalized gait (3-D angular kinematics of gait, Vicon) and paw placement (Catwalk, Noldus) patter and normalization of outcome measures for pain compared to corresponding data obtained from the untreated injured controls. Our data to date suggest that combination of Tm and simultaneous magnetic stimulation can be a safe and effective treatment modality for C-SCI induced spasticity and gait impairments. The combination therapy revealed a profound therapeutic reduction of spasticity toward pre-injury levels.

Disclosures: **J. Hou:** None. **R. Nelson:** None. **J. Watts:** None. **G. Mustafa:** None. **J. Joseph:** None. **D. Fantaccione:** None. **F.J. Thompson:** None. **P. Bose:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 523.09/OO12

Topic: C.09. Brain Injury and Trauma

Support: Craig H. Neilsen Foundation 280072 (S.H.)

DoD/CDMRP W81XWH-14-1-0605 (V.J.T.)

Title: Grafting embryonic raphe nuclei cells into a complete spinal cord injury site reestablishes serotonergic modulation of sympathetic activity and improves cardiovascular regulation

Authors: *S. HOU[1], T. SALTOS[1], T. CONNORS[1], K. C. ELDAHAN[2], A. G. RABCHEVSKY[2], P. LU[3,4], V. J. TOM[1];
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Abstract: Cardiovascular dysfunction often occurs after high-level spinal cord injury (SCI). Disrupting supraspinal vasomotor pathways (SVPs) affects resting hemodynamics and leads to autonomic dysreflexia (AD) under unpleasant stimulus. Recently, grafting neural stem cells (NSCs) from embryonic (E) brainstem into a high-thoracic (T) SCI site enhanced cardiovascular regulation. We hypothesize that graft-derived serotonergic (5HT) reinnervation of caudal autonomic neurons plays a critical role in the functional recovery. To test this, we transplanted NSCs from raphe nuclei (RN-NSCs) of E14 GFP transgenic rats into an acute, complete T4 SCI cavity of adult wild-type rats. SCI rats grafted with NSCs dissected from E14 spinal cord (SC-NSCs), that contain very few $5HT^+$ neurons, or SCI rats without a graft served as two controls. Biotinylated dextran amine (BDA) was infused bilaterally into the rostral ventrolateral medulla (RVLM) to anterogradely trace host SVPs. A radio-telemeter was implanted into the descending aorta to measure cardiovascular parameters. Following 8 weeks, resting mean arterial pressure (MAP) in rats with injury only was significantly lower than in naïve rats. Grafting RN-NSCs restored basal MAP to normal, pre-injury levels. On the other hand, resting MAP in SC-NSC rats was significantly lower than in either RN-NSC or naïve rats and was similar to that in ungrafted SCI rats. In 24 h hemodynamic recordings, we detected significantly fewer spontaneous AD events in RN-NSC or SC-NSC rats compared to injury only controls. During colorectal distension-induced AD episodes, the magnitude of MAP elevation was remarkably lower in both RN- and SC-NSC groups versus injury controls. Pharmacologically blocking 5HT2A receptors, mainly expressed on sympathetic preganglionic neurons (SPNs), with ketanserin reduced resting MAP and elicited a responsive heart rate (HR) increase in RN-NSC rats but not in SC-NSC rats or SCI controls. Moreover, spinal cord re-transection above the graft abolished all functional recovery in RN-NSC rats, indicating the necessity of supraspinal input. Histological analysis showed that GFP^+ grafted cells survived and integrated with host tissue. BDA-labeled SVPs regenerated into the grafts. We perceived $5HT^+$ neurons in the RN-NSC grafts and dense $5HT^+$ axonal bundles innervating caudal autonomic regions, i.e. SPNs and the gray commissure. Very few $5HT^+$ neurons and axons were found in the graft and caudal cord of SC-NSC rats. Thus, grafting RN-NSCs into a lesion of SCI reestablishes 5HT regulation of sympathetic activity and improves cardiovascular performance.

Disclosures: S. Hou: None. **T. Saltos:** None. **T. Connors:** None. **K.C. Eldahan:** None. **A.G. Rabchevsky:** None. **P. Lu:** None. **V.J. Tom:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Program#/Poster#: 523.10/OO13

Topic: C.09. Brain Injury and Trauma

Support: Department of Defense (SC140089)

Shriners Hospitals for Children # 86000

Title: Investigating the role of cervical propriospinal relays in re-establishing forelimb function after a spinal cord injury.

Authors: *I. S. SHEIKH, Y. LIU, K. M. KEEFE, X.-Q. TANG, G. M. SMITH;
Neurosci., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Propriospinal interneurons (PNs) are found throughout the spinal cord, forming simple and complex circuits between different spinal segments. PNs function by integrating supraspinal motor and sensory information to organize locomotion. Studies postulate that spared PNs can reorganize and contribute to functional recovery by establishing relays between lesioned axons and motor neurons. However, to what extent PNs contribute to recovery of forelimb reaching and grasping following SCI is not known in rodents. In our first study, we examined the number and distribution of supraspinal (cortico-, rubro- and propriospinal) neurons that innervate forelimb motoneurons in C6-T1 spinal levels. We utilized a lentivirus that permits highly efficient retrograde labeling of GFP (HiRet-GFP) to neuronal somas when injected at synaptic terminals near motoneurons. Histological analysis showed C3-C4 PNs bilaterally labeled with GFP in the grey matter with greater numbers ipsilateral to the injection. Rubrospinal neurons were labeled contralateral to the injection with sparse GFP+ neurons in the ipsilateral red nucleus. Very sparse GFP-labeled corticospinal neurons were observed in the contralateral cortex. In our second study, we tested if manipulation of the intrinsic axonal growth program of severed corticospinal tract fibers combined with neurotrophin expression from spared C3-C4 PNs can permit forelimb recovery after a cervical lesion. We promoted upregulation of the PI3K/Akt/mTOR pathway by injection of AAV2-shPTEN-GFP into the primary somatosensory cortex in adult rats several days prior to a C5 dorsolateral quadrant lesion. Immediately after the lesion, we injected HiRet-NT3 at PN synaptic terminals to permit neurotrophin-3 expression from spared interneurons rostral to the lesion. We observed limited recovery of the forelimb in the IBB and grip-strength test. No recovery was observed in the cylinder and single-pellet reaching test. We intend to further explore whether propriospinal relays can influence forelimb functional recovery post injury by expressing inhibitory DREADDs in C3-C4 PNs. We will also examine if changes in axonal sprouting with therapeutic intervention can promote increased plasticity from severed corticospinal fibers onto propriospinal relays.

Disclosures: **I.S. Sheikh:** None. **Y. Liu:** None. **K.M. Keefe:** None. **X. Tang:** None. **G.M. Smith:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Program#/Poster#: 523.11/OO14

Topic: C.09. Brain Injury and Trauma

Support: Department of Defense (SC140089)

Shriners Hospitals for Children # 86000

Title: Mapping propriospinal interneurons and their lumbar connections in a contusive injury model

Authors: ***K. M. KEEFE**, Y. LIU, C. ENEANYA, N. STERLING, G. SMITH; Neurosci., Temple Univ., Philadelphia, PA

Abstract: Spontaneous recovery from a contusive spinal cord injury is thought to involve mechanisms such as remyelination, recovery from spinal shock, and sprouting of damaged axons onto intact circuitry to form adaptive pathways. One likely target of sprouting axons is propriospinal interneurons (PNs), a ubiquitous population of neurons that reside in the intermediate grey matter of the spinal cord. These neurons receive and integrate signals from motor tracts that control locomotion such as the corticospinal, rubrospinal and reticulospinal tracts. One particular group of PNs, the long descending propriospinal neurons (LDPNs), are especially interesting to study after a contusive injury as their axons traverse the lateral funiculi of the spinal cord, and thus represent a potentially preserved population of descending axons. This makes them a likely contributor to spontaneous recovery. As this has never specifically been studied, we first focus on mapping LDPNs with axons bypassing a contusive injury, their supraspinal connections and their connections onto lumbar neurons. 200kD contusions are performed on female Sprague Dawley rats at spinal level T10. Four weeks later, we inject HiRet-GFP lentivirus into the lumbar region for retrograde tracing to the brain and spinal cord. GFP positive neuronal counts were performed on the cortex, red nucleus, medullary and pontine reticular formation, raphe nuclei, locus coeruleus, and lateral vestibular nuclear areas of the brain, and the thoracic and cervical spinal cords. To track supraspinal connections onto LDPNs, we additionally inject AAV-mCherry-WGA into the red nucleus, pontine reticular formation, or medullary reticular formation. This allows us to quantify percentages of neurons bypassing the injury in each pathway, identifying which supraspinal pathways might be involved in the

formation of adaptive circuitry. Future studies will target these populations with synaptic silencing techniques to verify their role in spontaneous behavioral recovery.

Disclosures: K.M. Keefe: None. **Y. Liu:** None. **C. Eneanya:** None. **N. Sterling:** None. **G. Smith:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

Location: Halls B-H

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Program#/Poster#: 523.12/PP1

Topic: E.09. Spinal Cord Injury and Plasticity

Title: Effects of transcutaneous spinal cord stimulation on soleus H- and posterior root motor reflexes: A pilot study

Authors: *B. FARRELL[1], K. HUANG[2], J. JACKSON[1], N. KAUSAR[1], I. SHVARTSMAN[1]; [1]Physical Therapy, [2]Georgia State Univ., Atlanta, GA

Abstract: Studies have shown that spinal cord stimulation (SCS) can decrease spasticity, specifically hyperreflexia, in individuals with spinal cord injury. Traditional epidural SCS is invasive and costly however, similar effects on spasticity using transcutaneous spinal cord stimulation (tSCS) have been seen. In addition, the electrode placement for tSCS can be used to elicit posterior root muscle (PRM) reflexes, which are thought to be analogous to the soleus H-reflex and provide additional insight into the effects of tSCS. Therefore, the purpose of this study was to evaluate how lumbar tSCS alters in the soleus H- and PRM reflexes. Based on the reported data, our hypothesis was that tSCS will reduce reflex amplitude and will demonstrate an enduring effect. Eight healthy subjects with no previous neurological damage were recruited for the study. Each subject had EMG electrodes placed over select muscles in the legs. Stimulation was provided in the popliteal fossa for eliciting H-reflexes and over the T11/T12 spinous processes for eliciting PRM reflexes. Each subject underwent baseline reflex testing followed by 15 minutes of tonic tSCS through the same electrodes on the back. Reflexes were reassessed during (H- only), immediately after, and 10 minutes after the bout of stimulation.
Of the eight subjects, five showed a decrease in peak-to-peak soleus H- reflex amplitude (10% decrease) while one showed no change and two increased (14% increase) immediately after tSCS. After the washout period, we found H-reflexes were reduced in six of the eight subjects (28% decrease), while the same 2 subjects remained elevated 9%). For the PRM reflex the results were different. Only four of the eight showed a decrease but the decrease was greater than that of the H-reflex (51% vs 10% decrease) immediately after the bout of tSCS. The remaining subjects showed an increase (9.05%).

These results provide preliminary evidence in support our hypothesis that tSCS can modulate reflexes with a lasting effect, though larger samples are needed. Interestingly, while the PRM reflexes were less responsive within the group, the changes were significantly greater and the PRM reflex was often absent after tonic tSCS. This may suggests that these PRM reflexes represent activation of different populations of afferents, which are undergoing different modulation.. Future studies will attempt to increase the sample size as well as provide sham stimulation condition to confirm these results.

Disclosures: B. Farrell: None. **K. Huang:** None. **J. Jackson:** None. **N. Kausar:** None. **I. Shvartsman:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Program#/Poster#: 523.13/PP2

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH Grant NS17323

Title: Spinal neurons from adult mice do not show enhanced excitability after spinal cord injury in reduced calcium saline and elevated temperature

Authors: *B. R. JOHNSON[1], K. LETT[2], S. DIETZ[2], A. HUSCH[3], R. M. HARRIS-WARRICK[2];
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Abstract: Spinal motor neurons and interneurons from neonatal rodents show enhanced excitability and bistable firing properties when recorded in temperatures above 30°C and using saline with 1.2 mM calcium, approximating normal in vivo conditions (Bouhadfane et al 2013. J Neuroscience, 33:15626; Brocard etal 2013. Neuron 77:1047). This increased excitability is thought to contribute to locomotor rhythm generation and plateau properties of spinal neurons. We are investigating the intrinsic firing properties of lumbar motor neurons (MNs), V1 interneurons (INs), and V2a INs from intact and spinal cord injured (SCI) adult (at least 8 weeks old) mice, comparing 2.5 to 1.2 mM Ca and 20 to 30°C, in order to optimize experimental conditions to detect excitability differences between spinal neurons recorded from intact and SCI mice. Perforated and whole cell patch recordings were made from identified lumbar MNs in longitudinal spinal cord slices from ChAT-GFP transgenic mice; V1 and V2a INs were recorded in transverse slices from En1-RFP and Chx10-CFP mice. Most neurons were pharmacologically

isolated from fast synaptic input. Excitability was measured with depolarizing current steps and ramps, and bistability was detected by negative hysteresis on ramps, or prolonged after-discharges following a current step. At 30° C and in 1.2 mM Ca saline, action potential firing in MNs and INs from intact adult mice was generally limited to the depolarizing phase of a symmetrical current ramp current; bistability was not detectable. After SCI, we have only examined excitability in MNs, 4 to 6 weeks after transection of the thoracic spinal cord, with the higher temperature and low calcium saline. MNs from SCI mice did not show signs of enhanced excitability or bistability, confirming our earlier work on MN properties at room temperature and higher calcium concentration. Some SCI MNs even expressed reduced excitability when shifted to higher temperature and 1.2 mM Ca, compared to room temperature and 2.5 mM Ca saline. SCI mice showed rear leg paralysis and other behavioral deficits consistent with complete SCI. Motor neurons from SCI mice were 100-1000-fold more sensitive to serotonin than control MNs, as found previously for V2a INs. These results suggest that the enhanced excitability seen under higher temperature and reduced Ca conditions in MNs and INs from neonatal mice may be lost during postnatal development, and SCI does not restore it, at least in MNs.

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Poster

523. Spinal Cord injury: Therapeutic Development

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Title: Dynamic electrical stimulation modulates spinal reflexes

Authors: *G. TACCOLA[1,4], P. GAD[1], C. CHANG[2], H. ZHONG[1], W. LIU[2], V. EDGERTON[1,3];
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Abstract: Electrical stimulation of spinal structures represents one of the most promising strategies to restore locomotor functions following neuro-motor disturbances. Animal studies have been useful to assess the best parameters of stimulation intensity, frequency and localization. Nevertheless, as of today, only stereotyped trains of square pulses have been used, while different stimulating patterns and their consequences on locomotion have not yet been explored. However, several studies suggest that critical levels of variable stochastic inputs are important for spinal networks to provide effective neural control, even during highly repetitive motor tasks. Using an *in vivo* rat model and exploiting an innovative array technology that allows independent recordings and stimulations, we concurrently delivered different patterns of intrinsically-variable bio-signals at weak intensity to distinct sites of the lumbosacral spinal cord. We named this innovative approach *dynamic neuromodulation* to distinguish it from canonical stereotyped protocols. Indeed, our stimulating patterns consist in traces previously recorded either during real locomotion from hindlimb EMGs and dorsal cord potentials, or action potential barrage from single motoneurons. Dorsum potentials recorded from the spinal cord and EMG activity recorded bilaterally from the tibialis anterior and soleus muscles were continuously monitored through the concomitant delivery of single square pulses to the spinal cord, at varying intensities. Responses evoked by single pulses at threshold only and not at supra-threshold amplitude, stably increased during dynamic stimulation and remained higher throughout the following resting phase. Moreover, even sub-threshold stimuli elicited responses after about 10s of stimulation. In conclusion, multisite independent stimulation of the cord with weak and intrinsically variable bio-signals efficiently modulates spinal excitability, providing the rationale for exploring new strategies of dynamic neuromodulation to restore muscle leg recruitment after a spinal injury.

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Poster

523. Spinal Cord injury: Therapeutic Development

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 523.15/PP4

Topic: E.09. Spinal Cord Injury and Plasticity

Support: The State of Florida Department of Health

The Buoniconti Found

Title: Characterization of motor and somatosensory evoked potentials in the yucatan minipig using transcranial and epidural stimulation

Authors: *F. D. BENAVIDES[1], A. J. SANTAMARIA[1], N. BODOUKHIN[1], L. G. GUADA[1], Y. NUNEZ[2], J. P. SOLANO[2], J. D. GUEST[3];
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Abstract: Introduction: Electrophysiological assessments permit analysis of connectivity in the nervous system. They are a valuable outcome measure bridging animal to human studies. Yucatan micropigs have spinal dimensions similar to humans, useful for spinal cord injury (SCI) studies. Micropigs are readily trained in both open field and treadmill quadruped locomotion and exhibit graded recovery after thoracic SCI. However, they are ungulates, with little neurophysiologic description of their motor and sensory pathways reported. It was necessary to develop such methods and determine normative values. Further, the relative importance of motor pathways such as the corticospinal tract (CST) in pigs must be discerned. We explored the hypothesis that motor and sensory organization would be similar to humans. In addition, we conducted studies based on motor and sensory stimulation and recording from paired epidural electrodes spanning sites of proposed SCI. **Methods**: Juvenile micropigs, trained to both treadmill and open field locomotor tasks were used. Protocols to evoke motor (MEP) and record sensory evoked potentials (SSEPs) from the scalp and spinal surface were developed in anesthetized uninjured animals. Optimal anesthetic conditions were determined to maximize sensitivity and waveform amplitude and consistency. SSEPs were evoked by stimulating peripheral nerves. The cortex was stimulated electrically using trains of pulses and EMG recorded from all limbs. Following laminectomy, epidural electrodes were placed at T8 and T10. **Results**: The response detection frequency, latencies, amplitudes, and variability of evoked potentials were determined. SSEPs were reliable and best detected during stimulation of peripheral nerve and epidural stimulation by referencing the contra-lateral cortex to midline point (Fz). The most reliable hindlimb MEP occurred in tibialis anterior. An inter-limb propriospinal pathway was discovered. Autopsy and direct cortical stimulation studies revealed variations in cortical morphology and optimal stimulation points between animals. Cervical D waves were observed, but stimulation of muscles required multiple pulses, suggesting polysynaptic circuits

were needed to recruit motorneurons. **Conclusions**: This EP study establishes neurophysiologic measures for the Yucatan minipig. Epidural stimulation, while less specific, is more potent to trigger EMG signals. The motor pathway organization requires further study to determine the role of the CST and other systems for limb activity. Yucatans differ from humans in the extent of cortical representation and the pathway to activate motor neurons.

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Poster

523. Spinal Cord injury: Therapeutic Development

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: DoD SC090469

Neilsen Foundation #284874

Neilsen Foundation #297076

Title: Manipulating chronic inflammation and neural plasticity away from the site of rodent spinal cord injury

Authors: *K. E. TANSEY[1], H. LEE[2], J. CHUNG[2], M. TANSEY[3];
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Abstract: Lateral hemisection spinal cord injury (SCI) at T10 produces nociceptive hypereflexia in the cutaneus trunci muscle (CTM) reflex and the sprouting of nociceptive afferents in dorsal cutaneous nerves (DCNs), the afferent limbs of the reflex, 6 weeks after SCI, both above (T7) and below (T13) the level of injury, on both sides of the spinal cord. The numbers of Iba1+ microglia/macrophages, but not GFAP+ astrocytes, are also increased at T7 and T13 at this chronic time point following T10 SCI. Because a persistent inflammatory environment following SCI is thought to be related to the development of chronic neuropathic pain, we hypothesized that a selective soluble Tumor Necrosis Factor (TNF) blocker, XPro1595, could modulate chronic inflammation in these spinal segments away from the injury and alter the neural plasticity seen there. XPro1595 has been shown to impact inflammatory cell biology and could impact neural transmission by blocking microglial-derived TNF effects on glutamate and GABA

receptors. Long Evans female rats (n=19) were subjected to a T10 lateral hemisection SCI and injected with either 3 mg/kg or 10 mg/kg of XPro1595 subcutaneously every third day starting the day of surgery. Therapeutic levels of XPro1595 were detected in plasma, cerebrospinal fluid (CSF), brain, and spinal cord segments (T7, T10, T13) 2 weeks after SCI in a dose-dependent manner. Additional animals (n=8) were then treated at 10 mg/kg for 6 weeks following SCI. This chronic XPro1595 treatment reduced the number of Iba1+ microglia/macrophages at T7 to uninjured levels and at T13 to lower than uninjured levels 6 weeks after T10 SCI. XPro1595 treatment also reversed injury induced nociceptive hyperreflexia, returning T7 DCN evoked CTM reflex sizes to uninjured values and causing hyporeflexia relative to the uninjured state in T13 DCN evoked reflexes. The effect of 6 weeks of XPro1595 on nociceptive afferent sprouting in T7 and T13 DCNs is being evaluated. Gene expression levels were also investigated at T7 and T13 in uninjured, T10 SCI, and T10 SCI with XPro1595 treatment animals using qRT-PCR arrays. Groups of genes related to inflammation, neurotrophism, neural transmission, synaptic plasticity, and myelination are being evaluated in particular. This study is the first we know of to examine the relationships between inflammation, reflex physiology, afferent anatomy and genetic changes associated with neural plasticity in the spinal cord away from the site of injury.

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Poster

523. Spinal Cord injury: Therapeutic Development

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NICHD R15HD075207

ARL 67444-LS-H

Title: Multielectrode array studies with culture models of motor neurons

Authors: ***A. THARANEETHARAN**[1], S. K. CUSTER[2], M. A. HARRINGTON[1];
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Abstract: Electrophysiological studies of motor neurons have frequently been approached using rodent models, and usually viewed from the single cell basis of a patch clamp. Recent work has shown that both embryonic chick spinal neurons and NSC-34 (a motor neuron hybridoma) are both robust models with little previous literature on their cellular electrophysiological characteristics, let alone network wide investigation using multielectrode arrays. Due to the

technically difficult and financially strenuous nature of murine models in spinal motor neuron culture, establishing an acceptable primary and secondary culture model via chick and NSC-34 would be of great value for smaller research institutions investigating motor neuron diseases such as Spinal Muscular Atrophy (SMA) or Amyotrophic Lateral Sclerosis (ALS). Spinal ventral horn neurons from embryonic chicks and NSC-34 cells were cultured onto probes for Alpha Med Scientific's MED64 & Axion's 64 electrode Muse system. Spontaneous firing generally appears in both models after 1-2 weeks while synchronized firing appears around 3-4 weeks. Pharmacologic evidence for the presence of active cholinergic and glutamatergic synapses through the use of receptor specific antagonists, such as atropine, oxotremerine, and mecamylamine (acetylcholine receptors) and CNQX and APV (glutamate receptors) were used in combination with optogenetic stimulation. Cholinergic and glutamatergic receptor regulation with myocyte cocultures was also examined. Co-cultures were done with both activated and inactivated astrocytes to mimic reactive gliosis and central nervous system pathology, while studying changes in network synchronicity.

Disclosures: A. Tharaneetharan: None. **S.K. Custer:** None. **M.A. Harrington:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Topic: C.09. Brain Injury and Trauma

Support: NRF-2014R1A2A1A11052042

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Title: Elucidation of gene expression pattern in the Brain after Spinal Cord Injury

Authors: *A. BAEK[1,2], M. KIM[2,3], J. SEO[2,3], S. WI[2,3], S. KIM[1], S.-R. CHO[2,3];
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Abstract: Introduction: Spinal cord injury (SCI) is a devastating neurological disease without effective treatment. SCI induced hyperpathia for sustaining neuroinflammation in brain regions regulating pain sensation. But this issue has not been addressed mechanistically. To generate a comprehensive view of the mechanisms involved in brain after spinal cord contusion, we applied

transcriptome analysis to characterize the temporal changes in global gene expression pattern in SCI mouse model.
Methods: This study sequenced brain samples in three groups from sham control, acute and subacute phases (3hrs and 2wks after injury). The samples from the three groups systematically characterized the transcriptome analysis. Differentially expressed genes (DEG), the result of transcriptome analysis, was then analyzed by a program Database of Annotation Visualization and Integrated Discovery (DAVID), which yielded Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.
Results: The top enriched pathways included oxidative phosphorylation, immune-related pathways. Oxidative phosphorylation was related to the acute model, and immune-related pathways were related to the subacute model. Comparing between subacute phase and acute phase, MAPK signaling pathway was shown as the significantly relative result.
Conclusion: Since following pathways—Oxidative phosphorylation, immune-related pathways and MAPK signaling pathway—have been known as the relative pathways in SCI, when this study found the same pathways at the brain in the SCI model, we suggested the relevance between SCI and brain injury. These results provide a valuable reference data for the better understanding in the brain injury with SCI in acute and sub-acute phases.
Acknowledgements: This study was supported by grants from the National Research Foundation (NRF-2014R1A2A1A11052042, NRF-2015M3A9B4067068), the Ministry of Science and Technology, Republic of Korea.

Disclosures: **A. Baek:** None. **M. Kim:** None. **J. Seo:** None. **S. Wi:** None. **S. Kim:** None. **S. Cho:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 523.19/PP8

Topic: C.09. Brain Injury and Trauma

Title: Botulinum toxin type a for neuropathic pain in patients with spinal cord injury

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Abstract: Objective: To evaluate the analgesic effect of botulinum toxin type A (BTX-A) on patients with spinal cord injury-associated neuropathic pain. **Methods:** The effect of BTX-A on 40 patients with spinal cord injury-associated neuropathic pain was investigated using a

randomized, double-blind, placebo-controlled design. A one-time subcutaneous BTX-A (200 units) injection was administered to the painful area. Visual analogue scale scores (0-100 mm), the Korean-version of the short-form McGill Pain Questionnaire, and the World Health Organization WHOQOL-BREF quality of life assessment were evaluated prior to treatment and at 4 and 8 weeks after the injection. **Results:** At 4 and 8 weeks after injection, the VAS score for pain was significantly reduced by 18.6 ± 16.8 and 21.3 ± 26.8, respectively, in the BTX-A group, whereas it was reduced by 2.6 ± 14.6 and 0.3 ± 19.5, respectively, in the placebo group. The pain relief was associated with preservation of motor or sensory function below the neurological level of injury. Among the responders in the BTX-A group, 55% and 45% reported pain relief of 20% or greater at 4 and 8 weeks, respectively, after the injection, whereas only 15% and 10% of the responders in the placebo group reported a similar level of pain relief. Improvements in the score for the physical health domain of the WHOQOL-BREF in the BTX-A group showed a marginal trend toward significance ($p = 0.0521$) at 4 weeks after the injection. **Conclusion:** These results indicate that BTX-A may reduce intractable chronic neuropathic pain in patients with spinal cord injury.

Disclosures: M. Chung: None. **Z. Han:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 523.20/PP9

Topic: C.09. Brain Injury and Trauma

Title: Analysis of cortical plasticity after spinal cord injury using resting state-fMRI in awake adults mice

Authors: *K. MATSUBAYASHI[1], A. IWANAMI[2], N. NAGOSHI[2], Y. KOMAKI[3], M. MATSUMOTO[2], N. TAKATA[2], H. OKANO[2], M. NAKAMURA[2];
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Abstract: [Purpose] It is recently reported that after spinal cord injury (SCI), neuronal connectivity changes occur not only in the spinal cord, but also in the brain. However, there has been no report investigating the changes of the neuronal functional communication among the regions of the cerebral cortex after SCI .The purpose of this study is to clarify the changes in neuronal functional connectivity of the brain after SCI by taking the resting state-fMRI (rs-fMRI).[Method] C57BL6 female mice were subjected to rs-fMRI without anesthesia. After careful acclimation to environmental stress, rs-fMRI was performed and the data of different

mice brain were standardized with the stereotaxic MRI brain template. By classifying the regions of the brain based on the Allen mouse brain atlas, the neuronal functional connectivity was analyzed among the specific regions. Next, complete transection or contusion SCI was induced at Th10 level in these mice. rs-fMRI was taken at 1,3,7 and 14 weeks after injury and the changes in neuronal functional connectivity of their brain were visualized using SPM12 software and CONN toolbox, and these data were analyzed using graph theory.[Results] First, the normal neuronal functional connectivity was successfully detected in the brain of the awake mice through rs-fMRI., In the comparative analyses before and after the transection SCI, the changes in functional connectivity was observed between the primary and secondary motor cortex. Moreover, by analyzing the brain connectivity of the contusion injury models, we detected the neuronal functional connectivity changes that accompanied motor recovery. Then, the data were analyzed using the graph theory and the network density of each brain region was quantitatively evaluated. The density of the network in the entire brain tended to decrease after SCI, and regarding the community structure in the network, the number of community and a region of the brain to constitute the community change time by time after SCI.[Conclusion] In the current study, we demonstrated the feasibility to take and evaluate the rs-fMRI of mice. We also showed the relative changes in the neuronal functional connectivity in the brain after SCI and identified the regions that were strongly related to the functional recovery after SCI. In addition, we detected that the networks changed in the brain after SCI by the analyses using a graph theory. These networks were divided into small communities and the density of these networks decreases immediately after SCI, then individual regions change the importance of their network, suggesting that brain networks were reorganized and changed the efficiency of the entire network after SCI.

Disclosures: **K. Matsubayashi:** None. **A. Iwanami:** None. **N. Nagoshi:** None. **Y. Komaki:** None. **M. Matsumoto:** None. **N. Takata:** None. **H. Okano:** None. **M. Nakamura:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Topic: C.09. Brain Injury and Trauma

Support: Health Canada

Western Economic Diversification Canada

Government of British Columbia

Government of Ontario

Title: Serum inflammatory cytokines and biomarkers of injury severity in acute human spinal cord injury

Authors: *K. DONG[1], F. STREIJGER[1], L. BELANGER[3], L. RITCHIE[3], S. PAQUETTE[2], J. STREET[1,2], T. AILON[2], M. BOYD[2], C. G. FISHER[2], M. F. DVORAK[1], N. MANOUCHEHRI[1], K. SO[1], B. K. KWON[1,2];
[1]Intl. Collaboration on Repair Discoveries, [2]Orthopaedics, Univ. of British Columbia, Vancouver, BC, Canada; [3]Vancouver Spine Program, Vancouver Gen. Hosp., Vancouver, BC, Canada

Abstract: Neurologic impairment after spinal cord injury (SCI) is currently measured and classified by functional examination. Biological markers that objectively classify injury severity and predict outcome would greatly facilitate efforts to evaluate acute SCI therapies. We recently reported that concentration levels of inflammatory (IL-6, IL-8, and MCP-1) and structural proteins (TAU, S100B, and GFAP) within the cerebrospinal fluid (CSF) of acute SCI patients are distinct between different injury severities (AIS A, B, and C) and could be utilized to predict AIS conversion and motor score recovery at 6 months post-injury. In an attempt to identify blood protein biomarkers and whether CSF changes are reflected in blood, in this study we conducted similar biochemical analyses in serum samples of SCI patients for whom we have already measured the CSF.

Patients with cervical or thoracic SCI classified as AIS A, B, or C were recruited for insertion of a lumbar intrathecal catheter. Blood and CSF samples were drawn at the same time at the time of catheter insertion and then in the subsequent post-operative period approximately 3 times each day. Blood samples were kept at room temperature for 20 minutes and then centrifuged at 10,000 rcf for 5 minutes to obtain serum. Serum concentrations of IL-6, IL-8, TNF-R1, MCP-1, and IP-10 levels were evaluated using a custom 5-plex kit. Beads were read on the Bio-Plex suspension array system. Serum levels of tau, S100β and GFAP were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits.

Initial analysis of serum samples from 9 acute SCI patients revealed that the concentrations of proteins such as IL-6, GFAP, and S100β were many orders of magnitude lower in serum when compared to CSF. This is, however, not to say that biomarkers within blood may not correlate with injury severity or predict outcome. Data analysis of serum samples from a small set of AIS A, AIS B, and AIS C patients collected 17-23 hours post-injury showed great promise and revealed an injury severity-dependent change in expression levels of TNF-R1, IL6, MCP-1, IP-10, and GFAP. Notably, increasing the overall sample size of the present study is necessary. This work is currently being done.

By characterizing the acute pathophysiologic responses to traumatic SCI in humans and establishing serum biomarkers for better injury stratification and improved prognostication of neurologic recovery, we hope to provide the field with tools that can be utilized in clinical trials to facilitate the evaluation of novel therapies.

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Poster

523. Spinal Cord injury: Therapeutic Development

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Topic: C.09. Brain Injury and Trauma

Support: NIH Grant R01NS054734

Craig H. Neilsen Foundation

Wings for Life

Title: Improved assessment of functional recovery after pyramidotomy injury in mice: Optimized staircase reaching task

Authors: *J. M. MEVES[1,2,3], C. G. GEOFFROY[1], J. J. KIM[1], X. LI[1], B. ZHENG[1,2];
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Abstract: The unilateral pyramidotomy model is a valuable tool for assessing compensatory growth of uninjured corticospinal tract (CST) axons after damage to the central nervous system. In this model, one side of the CST is lesioned at the level of the medullary pyramids, which induces spontaneous growth of the contralateral intact CST axons in the denervated side of the spinal cord. Previous studies have shown that this form of axon growth can be increased by manipulating a variety of neuron-intrinsic or extrinsic axon growth regulators. While this model allows clear anatomical assessment of axon growth after injury, the use of this model in mice has been hampered by a lack of consistent and robust behavioral assessments. Here we show that a modified staircase reaching task reveals a clear, persistent functional deficit after a unilateral pyramidotomy injury in mice. This behavioral paradigm requires minimal hands-on training of animals or subjective scoring of performance, lending itself as a valuable tool for ongoing studies that assess the functional relevance of molecular manipulations that promote compensatory axon growth.

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Poster

523. Spinal Cord injury: Therapeutic Development

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Topic: C.09. Brain Injury and Trauma

Support: Morton Foundation

Wings for Life contract # WFL-US-015/15

Title: The role of the RGD binding motif in the survival of a bone marrow-derived mesenchymal stem cell transplant in the injured spinal cord

Authors: *A. E. HAGGERTY, M. OUDEGA;
The Miami Project, Univ. of Miami, Miami, FL

Abstract: Spinal cord injury (SCI) results in nervous tissue loss and so far untreatable functional impairments. Preclinical studies have demonstrated that a transplant of bone marrow-derived mesenchymal stem cells (MSCs) elicit anatomical repair and partial functional recovery, likely through the secretion of paracrine factors. Previously, we have shown that the incomplete repair by an MSC transplant is in part due to their limited survival in damaged spinal cord nervous tissue (Ritfeld et al., 2014). MSCs are anchorage-dependent cells and therefore susceptible to anoikis (i.e., programmed cell death due to lack of adherence to a substrate), which at least in part could be responsible for their poor survival following transplantation into the damaged spinal cord. It is known that MSCs adhere via integrin receptors to the tripeptide, arginine-glycine-aspartic acid (RGD) which is present on components of the extracellular matrix, including fibronectin (FN). Therefore, we have investigated the effect on MSC survival of superfibronectin (sFN), a specific form of fibronectin with increased availability of RGD binding motives, as a cell suspension matrix both in vitro and in vivo using an adult rat model of spinal cord contusion. We have also combined sFN with a synthetic polyurethane-polyethylene glycol co-polymer hydrogel (ESHU), which was found to promote MSC survival in vitro and in vivo, likely due to anti-oxidative properties. We argued that this combination could lead to synergistic effects on MSC survival, with ESHU providing protection against reactive oxygen species-driven apoptosis and sFN providing protection against anoikis-driven apoptosis. This work is supported by the Morton Foundation and the Wings for Life Foundation (contract # WFL-US-015/15).

Disclosures: A.E. Haggerty: None. **M. Oudega:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Topic: C.09. Brain Injury and Trauma

Support: NSERC

FRQS

Fondation chiropratique du Québec

Title: Impact of sustained sublesional nociception on restoration of spinal excitability following complete spinal transection in mice

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Abstract: Pain affects two thirds of patients following spinal cord injury (SCI). While its psychosocial impact is well-understood, we just begin to unravel the interaction between nociception and sensorimotor adaptation responsible for functional improvement. Recent experimental evidence show that nociceptive afferents can hinder locomotor reexpression in mice with complete spinal transection. Besides, locomotor training, partially overcomes nociception-induced locomotor deficits. While part of training's adaptive effects on recovery is due to the recovery of spinal excitability, it is currently unclear how nociception induces its maladaptive impact on locomotor reexpression. The aim of this study is to examine if nociception triggered by chronic inflammation of sublesional paraspinal muscles by injecting complete Freund adjuvant (CFA) alters the recovery of H-reflex excitability following complete spinal transection in mice. H-reflex measurements were collected at day 28 following complete transection in mice and were compared between two groups, including mice receiving a CFA injection (CFA) and controls (CTL) receiving no injection. To avoid anesthesia-induced depression of spinal activity, mice were decerebrated before the recordings, which allowed collecting H-reflex measurements without anesthesia. Preliminary results suggest that H-reflex excitability, measured by the slope of the intensity-response curve, is decreased in CFA compared to CTL mice with complete spinal transection. Since H-reflex excitability is associated with better outcomes after SCI, it is possible that nociception alters the course of locomotor recovery by modulating spinal excitability.

Disclosures: R. Jeffrey-Gauthier: None. **M. Piché:** None. **H. Leblond:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Program#/Poster#: 523.25/PP14

Topic: C.09. Brain Injury and Trauma

Title: Decrease of M1 gray matter intensity contrast in Voxel-based morphometry are associated with somatic degeneration of pyramidal neurons in common marmosets with hemi-lesioned spinal cord

Authors: *K. SAKUYAMA[1], T. KONDO[3], Y. KOMAKI[4], K. YOSHINO-SAITO[3,6], F. SEKI[5], H. J. OKANO[7], E. SASAKI[4,8], H. OKANO[3], J. USHIBA[2];
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Abstract: Spinal cord injury (SCI) results in loss of voluntary motor control followed by incomplete recovery, which is partly mediated by the descending pathway from the primary motor cortex (M1). Human SCI study with magnetic resonance imaging (MRI) has reported that not only traumatic spinal degeneration but also disability-related atrophy of gray matter in M1 occurred in 2 months after the injury (Freund et al., 2013). However, yet actual cellular degeneration mechanisms behind its atrophic process has not been characterized in details. Therefore in the present study, we longitudinally investigated M1 atrophic process after SCI in common marmosets using voxel-based morphometry (VBM) of MRI gray matter density. After the detection of atrophy, we quantified the feature of shape and somatic volume of the layer 5 pyramidal neurons in M1 using SMI-32P staining, which labels neurofilament specifically expressed in layer 5 pyramidal neurons of cerebral cortex, and considered the relevance between the atrophy and the somatic volume of the pyramidal neurons in M1. Four adult female common marmosets underwent C4/C5 lateral hemisection. We acquired MR images at four time-points; before injury (baseline), and 2 weeks, 6 weeks, and 12 weeks after injury. We assessed atrophy in gray matter across the whole brain by VBM of T1-weighted MR images (N=3). We also performed immunohistochemistry of SMI-32P staining to characterize somatic volume of pyramidal neurons in the layer 5 of M1 at 12 weeks after SCI (N=2). MRI analysis showed a significant gray matter volume decrease in the contralesional M1, from baseline to 6 weeks after the injury [15%, Z score 5.87, $p<0.05$ (corrected for family-wise error)]. SMI-32P staining also revealed significant decrease in the somatic volume in the contralesional M1 compared with that in ipsilesional M1 (32%, two-sample t-test, $p<0.001$). This demonstrated that pyramidal neurons in the contralesional M1 have undergone significant shrinkage at least 12 weeks after SCI. The

current results indicate that gray matter volume decrease in contralesional M1 in hemi-spinalized common marmosets first occurs in 6 weeks after SCI, and at least in part, it results from shrinkage of pyramidal neurons. We showed, for the first time, features of time course of atrophic change of M1 from before the lesion through to the chronic stage. And it is expected that other multiple cortical structures such as microglia and astrocyte drastically made injury-induced reorganization. Therefore it is showed that, in order to clarify the whole picture of clinical condition after SCI, to reveal all those injury-induced drastic cellular reorganizations is important.

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Poster

523. Spinal Cord injury: Therapeutic Development

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Program#/Poster#: 523.26/PP15

Topic: C.09. Brain Injury and Trauma

Support: Department of Defense (DoD)

Title: Development of a novel technique to investigate the role of autonomic dysfunction in mechanical allodynia following spinal cord injury

Authors: *D. NOBLE, K. K. MARTIN, S. PARVIN, S. M. GARRAWAY;
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Abstract: Spinal cord injury (SCI) commonly results in the development of maladaptive pain and autonomic dysfunction. Because we do not fully understand the mechanisms of chronic and maladaptive pain after SCI, there has been limited success in controlling this pernicious facet of injury and developing new therapeutic interventions for its alleviation. Allodynia, defined as a painful response to innocuous stimulation, is indicative of maladaptive pain in studies using animal models. Behavioral tests commonly only assess allodynic responses distal to the dermatomal site of injury at the expense of at-level changes. Here, we present pilot data obtained from a novel setup designed to monitor rodent ventilatory responses - potentially a key autonomic index of at-level allodynia - to clinically relevant mechanical stimulation, with the aim of developing a more complete profile of clinical SCI pain. We focus on a specific fiber population, the C-fiber low-threshold mechanoreceptors (C-LTMRs). C-LTMRs can undergo injury-induced changes to produce allodynia and are of especial interest since it is an active research question whether they are involved in allodynia and chronic pain after SCI. We

stimulated adult mice (SCI with lateral T10 hemisection or sham) mechanically with a small brush at several frequencies within a range previously reported to activate C-LTMRs. At the same time, we used highly sensitive non-contact electric field sensors (EPIC, Plessey Semiconductors) to monitor the ventilatory response to stimulation. Breathing rates were elevated from baseline in SCI mice one day after injury, prior to the development of mechanical allodynia (assessed with the von Frey test), and remained elevated at later time points. Furthermore, mechanical brush stimulation across the animal's side at frequencies ranging from 0.3-30 Hz, corresponding to the tuning properties of C-LTMRs, revealed frequency-dependent changes in breathing rate. Together, these results suggest the possibility that pain is modulated by autonomic dysfunction following SCI and encourage further inquiry into the role of C-LTMRs. Ongoing studies are aimed at investigating breathing rate as a predictive autonomic physiological marker for below- and at-level SCI-induced pain, and selectively recruiting or inhibiting C-LTMRs using optogenetics to more precisely delineate their functional contribution to maladaptive pain following SCI.

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Poster

523. Spinal Cord injury: Therapeutic Development

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Basic Research Program(2015R1D1A1A02061196)

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Korea Health Technology R&D Project (HI14C-0522-010015)

Basic Research Program(2015R1D1A1A01059014)

Brain Korea 21 Plus Project(10Z20130012372)

Priority Research Centers Program(2009-0093829)

Title: Dna methylation in the brain following spinal cord injury

Authors: *J. HONG[1,2,3], J.-W. KIM[2,3], K. HONG[2], J. HYUN[2,3,4];
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Abstract: There are many therapeutic strategies to improve the functional restoration following spinal cord injury (SCI) experimentally, however the effects of treatments are limited and hard to be translated into the clinical setting. DNA methylation is one of the most important epigenetic modulator of gene expression in the mammalian, and recent studies reported that DNA methylation induced by spinal cord injury (SCI) may modulate regeneration-associated genes (RAGs) which control the regeneration capacity and neuronal growth. The aim of this study is to find epigenetic changes within a brain following SCI, and to delineate the supraspinal mechanisms of epigenetic modulation for the neuronal regeneration after SCI. Spinal cord contusion model at thoracic level was made using Sprague-Dawley rats, and performed immunohistochemistry dot blot, and real-time PCR to analyze 5-hydroxymethylcytosine (5hmc), 5-methylcytosine (5mc), Ten-eleven translocation (TET) family, RAGs, and pro-inflammatory genes within the brain from very acute stage (1 hour) to chronic stage (3 months) after SCI. The 5hmc intensity in the motor cortex of brain was significantly increased in SCI group more than in sham operated controls, and this change was prominent in the subacute stage. The mRNA levels of TET family (TET1, TET2, and TET3) were also increased within the brain from 1 hour until 3 months after SCI. Some of pro-inflammatory genes including interleukin-1 beta, interleukin-6 and tumor necrosis factor alpha were increased in the acute stage and many of RAGs were decreased from the subacute stage. We concluded that the activity of DNA methylation can lead to alteration of RAGs and inflammatory cytokines within a brain following spinal cord contusion in rats, and the epigenetic modulation at supraspinal level might be one of the therapeutic strategies for spinal cord regeneration.

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Poster

523. Spinal Cord injury: Therapeutic Development

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Korea Health Technology R&D Project HI14C-0522-010015

Brain Korea 21 Plus Project 10Z20130012372

Priority Research Centers Program 2009-0093829

Title: Time dependent changes of gene expression following spinal cord contusion in rats

Authors: *J.-W. KIM[1,2], J.-Y. HONG[1,2], J. HYUN[1,2,3];
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Abstract: Numerous clinical trials have been documented to promote the regeneration of damaged neurons after spinal cord injury (SCI), however no effective treatment is developed until now. The pathophysiology of SCI is extremely complex and many in vitro and in vivo studies have continued to report opposite results each other in spite of the same treatments, therefore more fundamental analysis such as an extensive assay of gene expression might be required to find a way for spinal cord regeneration. In this study, we aimed to detect the changes of global gene expression following spinal cord contusion in rats according to the time sequence, and to find a treatment target within the featured changes at each stage. We sequenced spinal cord tissues at contusion site after spinal cord contusion in rats using RNA-sequencing technology. Analyzed genes were classified into three categories according to the mechanisms of SCI; genes associated with inflammatory process, neuronal regeneration, or glial scar formation. For time sequence analysis, five time points was set; 1 hour, 1 day, 1 week, 1 month and 3 months after spinal cord contusion at T9 level using Infinite Horizon impactor, and sham operated rats were used as controls. Quantitative RT-PCR analysis was also performed to validate expression changes of candidate genes in each category. We found that the pattern of global gene expression at acute and subacute stages was quite different from that at chronic stage. Most of gene expression levels of inflammatory cell markers were increased and peak during acute stage (1 hour to 1 week) and maintained until chronic stage. Some of regeneration-associated genes (RAGs) including brain derived neurotrophic factor, glial cell derived neurotrophic factor and ciliary neurotrophic factor were increased at 1 hour or 1 day after SCI. The expression level of myelin associated genes such as APC, MBP, and PLP1 were all decreased from 1 day to 3 months, and most of gene expression levels of M1 macrophage-producing cytokines and chemokines increased from 1 hour after SCI and continue to chronic stage, however M2 macrophage-associated gene level was not changed after SCI. The expression changes of candidate genes using RT-PCR were consistent with RNA-sequencing results.We concluded that the information of gene expression level according to the time sequence after SCI might be useful to determine treatment strategies for spinal cord regeneration especially in chronic stage.

Disclosures: **J. Kim:** None. **J. Hong:** None. **J. Hyun:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

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Basic Research Program(2015R1D1A1A01059014)

Brain Korea 21 Plus Project(10Z20130012372)

Priority Research Centers Program(2009-0093829)

Title: aligned microchannel scaffolds for spianl cord repair

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Abstract: Axonal regeneration is hardly achieved spontaneously after spinal cord injury (SCI), and the restoration of somatic and autonomic functions after SCI is also challenging in the clinical field. We developed three dimensional scaffolds which contain numerus microchannels. The microchannels were created by dissolving finely aligned phosphate glass fibers within dried poly(lactic-co-glycolic acid) (PLGA)/tetraglycol or polycaprolactone (PCL)/tetraglycol solution, and the diameter of microchannel was about 20 micrometer. We aimed to delineate the effect of microchannel-containing biopolymer scaffolds to the axonal regeneration after SCI in rats. In vitro study was performed using a three dimensional culture system which enable neuronal cell culture within a scaffold, and we found that the neurite outgrowth of cortical neurons is more effective within PLGA scaffold than PCL scaffold. The PLGA or PCL scaffold were also implanted into transected spinal cord at T9 level in rats. Twelve weeks after implantation, outgrowing axons were visible within the PLGA or PCL scaffolds, and the number of axons crossing the scaffolds was significantly higher in PLGA scaffold than in PCL scaffold. We also applied neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF) to heparinized PLGA scaffold, and found that NT-3 and BDNF-applied scaffold is more effective to increase the amount of axonal outgrowth crossing the scaffold, and improve locomotor and bladder

functions 12 weeks after implantation. We concluded that PLGA-based mirochannels are effective for spinal cord repair and sustained release of NT-3 and BDNF within the scaffold also facilitate the axonal regeneration after spinal cord transection in rats.

Disclosures: D. Lee: None. **M. Kim:** None. **J. Kim:** None. **J. Knowles:** None. **J. Hyun:** None.

Poster

523. Spinal Cord injury: Therapeutic Development

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 523.30/QQ3

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH grant AG045656

Charles H. Smith Endowment Fund

Title: NeuroD1-mediated glia-to-neuron conversion in the injured spinal cord

Authors: H. LI, X. DING, A. CAI, B. PULS, M. KEEFE, K. HE, L. ZHANG, *G. CHEN; Biol., Penn State Univ., University Pk, PA

Abstract: Spinal cord injury (SCI) is a devastating neurological disorder that often impairs the daily function of patients for their entire life. Traumatic injury of the mammalian central nervous system (CNS) often leads to severe neuronal loss along with reactive gliosis, which remain irreversible with existing approaches. Stem cell-based transplantation strategy had great potential to replenish the lost neurons, but it faces ethical issues and the fact that transplanted cells are often eliminated by immune rejection. Recent advance in the field of *in vivo* reprogramming offers new hope to regenerate neurons from the endogenous glial cells. Our recent work showed that over-expressing a single neural transcription factor NeuroD1 in the stab-injured mouse brain converted reactive glial cells directly into functional neurons (Guo Z et al., 2014, Cell Stem Cell). In this study, we evaluate the same *in vivo* reprogramming technology in the adult spinal cord after a stab-injury. We found that NeuroD1 overexpression by a retrovirus was able to convert endogenous glial cells into NeuN+ neurons with high efficiency as early as 1 week after injection. Neurogenin2, a transcription factor upstream of NeuroD1, was also capable of neuronal conversion but with less efficiency than NeuroD1. Further immunostaining analysis showed that new neurons are likely converted from Olig2+ oligodendrocyte precursor cells (OPCs) but not GFAP+ astrocytes. In addition, both NeuroD1 and Neurogenin2 could efficiently reprogram both human and mouse spinal cord glial cells in cultures into functional neurons. Finally, NeuroD1-converted neurons seem to have a higher survival rate than the Neurogenin2-

converted ones. Our study suggests that NeuroD1 is capable of replenishing lost neurons by efficiently converting endogenous glial cells into neurons, providing a novel therapeutic treatment for SCI.

Disclosures: **H. Li:** None. **X. Ding:** None. **A. Cai:** None. **B. Puls:** None. **M. Keefe:** None. **K. He:** None. **L. Zhang:** None. **G. Chen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder of TCCT LLC.

Poster

524. Pain Models: Behavior

Location: Halls B-H

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Program#/Poster#: 524.01/QQ4

Topic: D.02. Somatosensation: Pain

Support: DoD Grant W81XWH1410579

Title: Characterization of nociceptive alterations and cognitive impairments in a preclinical model of polytrauma

Authors: ***P. SAHBAIE**[1,3], M. TAJERIAN[1,3], J. LUO[2,4], P. YANG[2,5], Y. SUN[1,3], T.-T. HUANG[2,5], T. WYSS-CORAY[2,4], J. CLARK[1,3];
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Abstract: Polytrauma consequent to war related injuries, falls or motor vehicle accidents involves injury to at least two body regions, commonly closed head concussive impact injuries (mild traumatic brain injury: mTBI) and limb injuries (bone fracture). mTBI accounts for about 80% of all TBIs, leads to chronic disability and is often accompanied by chronic pain. Chronic pain associated with mTBI is challenging to manage and hinders rehabilitation efforts. Specific treatments for TBI-related pain is lacking partly due to gaps in understanding underlying mechanisms of mTBI related chronic pain development. Many mTBI or chronic pain patients suffer long-lasting neurobehavioral impairments. Mice were assigned to the following experimental groups: Sham/Sham, mTBI/Sham, Sham/Fracture or polytrauma (mTBI/Fracture). Nociceptive sensitization to mechanical stimuli was measured and nociceptive priming effects of mTBI were evaluted using PGE2 challenge. Diffuse Noxious Inhibitory Controls (DNIC) on nociceptive sensitization was measured, immediately after forepaw capsaicin application. Also, Capsaicin evoked spontaneous pain behaviors were measured in all after resolution of

mechanical sensitization. Neurocognitive assessments were done by using the object location/recognition memory (OLM/ORM) tasks, after open field (OF) testing. Lateral closed cortical impact mTBI resulted in hindlimb mechanical allodynia lasting 7-14 days. The mTBI alone resulted in increased hyperalgesic priming and impaired descending pain modulation (DNIC) after local PGE2 application. The polytrauma group displayed more intense and prolonged mechanical allodynia compared to either mTBI or fracture groups. Additionally, impaired descending pain modulation (DNIC) was observed in polytrauma compared with fracture alone. Moreover, the combined injury group had increased sensitivity to local capsaicin when mechanical allodynia had resolved. Deficits in hippocampal dependent (NOL) and hippocampal independent working memory (NOR) were seen in both mTBI and polytrauma groups, though the latter displayed a more affected phenotype. Furthermore, mTBI and polytrauma groups had increased levels of activity (OF) indicative of agitation. Findings presented in our study provide novel observations regarding changes in pain and cognitive function in a mouse model of polytrauma. The model offers clinically relevant features useful for studying interactions between combined neurotrauma-induced pain and neurobehavioral changes with focus towards developing better therapeutic strategies. Further studies characterizing gender differences in the model need to be carried out.

Disclosures: **P. Sahbaie:** None. **M. Tajerian:** None. **J. Luo:** None. **P. Yang:** None. **Y. Sun:** None. **T. Huang:** None. **T. Wyss-Coray:** None. **J. Clark:** None.

Poster

524. Pain Models: Behavior

Location: Halls B-H

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Program#/Poster#: 524.02/QQ5

Topic: D.02. Somatosensation: Pain

Support: T32 COSTAR; DE14318

R01 GM106075

Title: Anoctamin 1 contributes to hypersensitivity associated with burn injury

Authors: *A. R. FURR, K. M. HARGREAVES;
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Abstract: Persistent pain associated with burn injury is a major clinical challenge primarily due the lack of efficacious analgesics. Although opioids are a gold standard for pain control, their efficacy in burn injury is insufficient and often associated with adverse side effects such as

tolerance and dependence. Thus, investigating mechanisms that underlie burn injury-related pain is critical for the development of novel, effective pain treatments with limited side effects.
To evaluate peripheral mechanisms of pain associated with burn injury, we have developed a mouse model of burn injury that encompasses the persistent hypersensitivities to thermal and mechanical stimulus modalities that are consistently reported clinically. Furthermore, we have identified a novel calcium-activated chloride channel, anoctamin 1, that contributes to persistent burn pain. We found that pharmacological inhibition of this channel attenuates both thermal and mechanical hypersensitivity on the day of peak burn injury-evoked pain.
To provide a neurophysiological correlate to burn injury-induced hypersensitivity observed behaviorally, we investigated spontaneous and stimulus-evoked response properties of sensory nerve fibers innervating burn-injured glabrous skin at peak nociception. Preliminary data suggest that pain-transducing sensory nerve fibers are activated at lower mechanical stimulation thresholds and display increased spontaneous firing frequency in burn-injured glabrous skin relative to sham controls.
The complementary behavioral and electrophysiological approaches provide a means to identify novel targets contributing to enhanced transmission of nociceptive signals and existence of ongoing burn injury-associated pain. Ongoing experiments will further characterize the stimulus-evoked and spontaneous discharge phenotype of sensory neurons at their peripheral terminals and how anoctamin 1 may contribute to burn injury-evoked alterations. Pharmacological modulation of this channel may serve as an effective treatment strategy for ongoing debilitating burn pain in patients.

Disclosures: **A.R. Furr:** None. **K.M. Hargreaves:** None.

Poster

524. Pain Models: Behavior

Location: Halls B-H

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Program#/Poster#: 524.03/QQ6

Topic: D.02. Somatosensation: Pain

Support: NINDS R01NS042595

NINDS R01NS069898

Title: Voluntary behaviors as readouts for persistent pain in mice

Authors: ***T. SHEAHAN**, E. R. SIUDA, A. J. SHEPHERD, D. P. MOHAPATRA, R. W. GEREAU, IV, J. P. GOLDEN;
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Abstract: Chronic pain represents an immense clinical problem, with over 100 million Americans afflicted and an annual price tag exceeding half a trillion dollars. One of the major hurdles to effective translation of basic pain research findings is the lack of research that directly translates from animal models into human studies. There is ongoing discussion regarding the validity of using only reflex/withdrawal measures of pain in animal studies, as these endpoints may not adequately model the complexities of human pain. The development of assays that reflect pain-induced changes in voluntary behaviors is therefore important in increasing the broad translatability of preclinical findings. Recently, voluntary wheel running, gait analysis, and social interaction have been used as endpoints to assess non-evoked pain responses in rodents. However, in some cases, it remains unclear if behavioral changes are due to pain or other comorbidities of injury (*i.e.* motor deficits). We tested voluntary wheel running, Catwalk automated gait analysis, and a social approach assay as measures of pain in the context of inflammatory and nerve injury-induced pain in adult C57BL6/J mice. When changes in voluntary behaviors were observed following injury, we tested whether such changes could be reversed by known analgesics. Supported by NINDS grants R01NS042595 and R01NS069898.

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Poster

524. Pain Models: Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 524.04/QQ7

Topic: D.02. Somatosensation: Pain

Support: R01NS086859

Title: Processing temperature stimuli: from receptors to behavior

Authors: *M. GALLIO;
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Abstract: Temperature affects nearly every biological process, hence it is not surprising that animals evolved sophisticated ways to sense and respond to temperature changes. How are hot and cold stimuli detected at the periphery? How are they processed in the brain? How are they integrated to produce behaviors such as temperature preference or avoidance of noxious extremes? We study temperature detection and processing using a variety of systems, from in vitro studies aimed at understanding the molecular mechanisms of heat detection, to whole-brain

imaging in the fruit fly Drosophila, a system ideally suited for the study of simple decision making and innate behavior.

Disclosures: M. Gallio: None.

Poster

524. Pain Models: Behavior

Location: Halls B-H

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Program#/Poster#: 524.05/QQ8

Topic: D.02. Somatosensation: Pain

Support: Grant-in-Aid for JSPS Fellows Number 16J02571

Title: A neuropeptide signaling regulates starvation-dependent reduction of heat nociception in *Drosophila*

Authors: *H. OHASHI, T. SAKAI;
Dept. of Biol. Sciences, Tokyo Metropoli, Hachioji, Tokyo, Japan

Abstract: Nociception is one of the most important sensory functions for animal survival. Many animals typically show escape behavior toward noxious stimuli. Feeding is also essential for animal survival, and hunger can modify various physiological functions. However, it remains unclear whether hunger affects nociception. Here, we report that wild-type *Drosophila melanogaster* shows the reduction of heat nociception after starvation. Immediately after wild-type flies are placed on the hotplate (≧44℃), they show jumping behavior to escape from noxious heat stimuli. To examine whether starvation can modify the responses to noxious heat, we used starved wild-type flies. After 6 and 12h of starvation, the number of flies showing the jumping behavior at 44℃decreased in comparison with that of non-starved flies. Thus, it is possible that 6-12h of starvation inhibits heat nociception.
We further established a novel behavioral assay of heat nociception using decapitated flies. Unlike the normal wild-type flies, the decapitated wild-type flies did not show jumping behavior toward noxious heat stimuli. However, they frequently showed tumbling behavior that gets up after falling on a hotplate. In contrast to the wild-type flies, the frequency of this behavior was significantly inhibited in painless mutant flies with defective heat nociception, indicating that the tumbling behavior can be used as the responses to noxious heat in decapitated flies. Using this behavioral assay, we next examined whether decapitated flies also show the starvation-dependent reduction of heat nociception. After 12h of starvation, decapitated wild-type flies showed normal heat nociception. Thus, it is possible that the reduction of heat nociception results from the starvation-dependent modification of brain functions.

In *Drosophila*, various neuropeptide signals influence feeding behavior because hunger/satiety state can be modified through neuropeptide release from neurosecretory cells in the brain. Thus, we next examined whether neuropeptides and their receptors involved in the regulation of feeding behaviors affect the starvation-dependent reduction of heat nociception. Mutant flies of *leucokinin* (*Lk*) did not show a significant reduction after 12h of starvation, suggesting that the Lk signaling can modify the responses to noxious heat. Since Lk acts as a full signal in *Drosophila*, it is likely that the reduction of Lk release in the brain triggers starvation-dependent reduction of heat nociception.

Disclosures: H. Ohashi: None. **T. Sakai:** None.

Poster

524. Pain Models: Behavior

Location: Halls B-H

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Program#/Poster#: 524.06/QQ9

Topic: D.02. Somatosensation: Pain

Title: Relationship between pain and anxiety in a model of facial carcinoma in rats.

Authors: *E. GAMBETA, C. M. KOPRUSZINSKI, R. C. DOS REIS, J. M. ZANOVELI, J. G. CHICHORRO;
Pharmacol., Federal Univ. of Parana, Curitiba, Brazil

Abstract: Orofacial cancer is highly incident in the world population. It is well known that pain and anxiety are common symptoms in this condition, and both significantly impair the quality of life of cancer-suffering patients. Pain control in cancer is considered highly unsatisfactory and associated with frequent and severe side effects. Moreover, clinical studies indicate that anxiety disorders are under diagnosed and undertreated in these patients population. For this reason, studies are warranted to better understand the relationship between cancer pain and anxiety and to provide new strategies that can help to control both symptoms. The aim of this study was to investigate the relationship between facial sensory changes and the anxiety-like behavior in rats with facial carcinoma. In addition, to investigate the influence of the anticonvulsant pregabalin, a drug with potential analgesic and anxiolytic-like effect, in both aspects. Facial carcinoma was induced by tumor Walker-256 cells subcutaneous inoculation in the right vibrissal pad of male Wistar rats. On the third and sixth day after inoculation, facial mechanical hypersensitivity was assessed by the application of a series of Von Frey filaments in the vibrissa pad, followed by animals analysis on the elevated plus maze, light-dark transition and open field tests. In a different set of experiments, the effect of pregabalin treatment (30 mg/kg, p.o.) was evaluated in all tests. All experimental procedures were previously approved by UFPR's Committee on the

Ethical Use of Animals (#938). Our results demonstrated that on day 3 after tumor cells inoculation, tumor-bearing rats showed facial mechanical hypersensitivity, but the anxiety-like behavior was not detected. On the other hand, on day 6 after tumor cells inoculation, tumor-bearing rats displayed facial mechanical hypersensitivity and anxiety-like behaviors.. In addition, systemic treatment with pregabalin was able to reduce facial mechanical hypersensitivity for 2 to 4 hours after its administration, and produced an anxiolytic-like effect when assessed in the EPM. In conclusion, our data indicate that facial tumor-bearing rats develop early mechanical hypersensitivity, which precedes the development of the anxiety-like behavior. Pregabalin may represent a useful drug in the treatment of both conditions. We thank CAPES for the financial support.

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Poster

524. Pain Models: Behavior

Location: Halls B-H

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Program#/Poster#: 524.07/QQ10

Topic: D.02. Somatosensation: Pain

Support: FAPESP 2014/15891-0

Title: Involvement of TRPV-1 receptors on thermal nociception in rats subjected to intraarticular persistent inflammation

Authors: *C. R. LEITE-PANISSI[1], J. K. NEUBERT[2], R. M. CAUDLE[2];
[1]Ribeirao Preto Dent. Sch. - USP, Ribeirao Preto, Brazil; [2]Univ. Of Florida, Gainesville, FL

Abstract: Orofacial pain has a high prevalence associated with debilitating disorders in modern society involving the neck, face, head and all intra-oral structures. In addition, temporomandibular joint (TMJ) -associated inflammation is considered to be one of the reasons for the pain reported by patients with temporomandibular disorders (TMD). Nevertheless, our previous study has shown that TMJ persistent inflammation promoted an anxiogenic-like effect in rats. To study orofacial pain mechanisms, a model using the operant behavior was developed and correlates orofacial pain with psychological processes. So, the aim of this study was to characterize thermal orofacial sensitivity (cold, neutral and hot stimuli) during development of TMJ using the operant behavior. In addition, we evaluated the role of transient receptor potential vanilloid 1 (TRPV1) on nociceptive responses of rats with TMJ persistent inflammation. The experiments were performed with hairless Sprague-Dawley rats (male and female, 250-300g)

with or without TMJ inflammation induced by carrageenan (CARR). The orofacial pain was evaluated on the operant orofacial pain assessment device (OPAD) in the presence of a range of cold, neutral and hot stimuli. Resiniferatoxin (RTX) was administered into TMJs prior CARR to lesion TRPV1-expressing neurons. Our results show that the number of facial contacts was significantly altered by the temperature of the thermodes in males and females with persistent TMJ inflammation. Particularly, we observed an increased number of facial contacts and alterations in the aversive index on neutral and cold temperature. However, the hot temperature (42°C) did not modify the parameters analyzed on the OPAD test (male or female rats). Additionally, the present results revealed that prior administration of RTX in the TMJ prevented the thermal hyperalgesia induced by CARR. In conclusion, the results show that the TMJ persistent inflammation altered the orofacial sensibility evaluated by OPAD. In particular, CARR administered into TMJ increased the number of face contacts and reduced the lick/face contact ratio (L/F) at cold (21°C) and neutral (37°C) temperatures in female and male rats. Additionally, our results show that at neutral temperature the hyperalgesia is longer in females compared to males. And, it was possible that TRPV1 is involved in this inflammatory hyperalgesia because the pre-administration of RTX into TMJ, that promote a pharmacological lesioning of TRPV1-expressing neurons, reduced these effects on male and female rats.

Disclosures: **C.R. Leite-Panissi:** None. **J.K. Neubert:** None. **R.M. Caudle:** None.

Poster

524. Pain Models: Behavior

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Program#/Poster#: 524.08/QQ11

Topic: D.02. Somatosensation: Pain

Support: GM 122747

UTHSCSA Dental School Pilot Project

Title: Postoperative pain in aged mice

Authors: M. PATIL[1], J. MECKLENBURG[1], *A. N. AKOPIAN[2];
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Abstract: Introduction: There is consensus that wound healing dynamics, biochemistry, physiology, and anatomy in the elderly is negatively altered by age (Benyamin et al., 2008; Portenoy et al., 2004). In addition, old age could affect plasticity in the nociceptive pathway. Very little basic research has been conducted on aged animals and some data contradict one

another (Yezierski, 2012; Weyer et al., 2016). Thus, it is unknown how old age affects postoperative pain. Accordingly, we have investigated whether postoperative thermal and mechanical hypersensitivity time-course and magnitude are altered in aged mice compared to adults. Given that hyperalgesic priming is an indicator of nociceptive pathway plasticity, we also evaluated whether age alters surgery-induced hyperalgesic priming. **Methods:** We used plantar incision model to model postoperative pain conditions in mice (Pogatzki and Raja, 2003). Thermal and mechanical nociception was measured using Hargreaves apparatus and Dynamic Plantar Aesthesiometer, respectively. Surgery-induced hyperalgesic priming was evaluated by PGE2 administration 10 and 24 days post-surgery for adults and aged mice, respectively. **Results:** Despite the fact that both 18-month-old and 24-month-old mice are considered "aged" their postoperative thermal and mechanical hypersensitivity was dramatically different magnitude and time-course-wise. Thus, adult (2-6 month-old) and 18 month-old mice have similar postoperative thermal and mechanical hypersensitivity levels and time-course (last 7-10 days). In contrast, postoperative thermal and mechanical hypersensitivity was more pronounced and lasted as long as 21 days for 24-month-old mice. Surgery-induced hyperalgesic priming was also statistically different in aged compare to adult mice. **Conclusion:** Postoperative hypersensitivity characteristics including hyperalgesic priming is dramatically altered by aging, especially in 24 month-old mice. This may indicate that neuronal plasticity is altered in the pain pathways in aged mice compared to adults. Therefore, pain management techniques will need to be adjusted in aged individuals.

Disclosures: **M. Patil:** None. **J. Mecklenburg:** None. **A.N. Akopian:** None.

Poster

524. Pain Models: Behavior

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Topic: D.02. Somatosensation: Pain

Support: NIH P01 GM113852

Wake Forest University School of Medicine

Title: Inflammatory abdominal pain disrupts performance in a visual attention task in rats.

Authors: *T. J. MARTIN, T. J. STRASSBURG, S. A. KIM, D. G. RIRIE, J. C. EISENACH; Anesthesiol., Wake Forest Univ. Sch., Winston Salem, NC

Abstract: Pain is a multisensory experience that produces behavioral effects across sensory/discriminative, affective/motivational, and cognitive domains. In laboratory animals, much work has focused on the sensory aspects of pain, with the affective/motivational and cognitive domains having received greater attention of late. Pain is known to interfere with tasks requiring sustained attention in humans, however similar effects of pain in laboratory rodents have not been well documented. We recently developed a variant of the 5 choice serial reaction time task in which task difficulty is systematically adjusted in real time to match the performance capabilities of the subject. This is achieved by altering the visual cue duration, such that cue duration decreases following correct responses and increases following incorrect responses or omissions. The sensitivity of this behavior in rats to inflammatory abdominal pain was examined using intraperitoneal injections of dilute lactic acid. The primary endpoint of visual attention performance was the median cue duration (MCD), which was increased in a linear manner (r^2=0.95) by injection of lactic acid concentrations ranging from 0.9% - 5.4% (v/v, 1 ml/kg i.p.). The MCD increased from 0.6±0.1 s at baseline to a maximum of 30±2.5 s after injection of 5.4% lactic acid. Pretreatment with either morphine (0.3-3.0 mg/kg, s.c.) or ketoprofen (0.03-0.3 mg/kg, s.c.) dose-dependently blocked the effects of 1.8% lactic acid injection on visual attention performance. Injection of scopolamine (0.03-0.3 mg/kg) also increased MCD in a dose-dependent manner, however pretreatment with either morphine (3.0 mg/kg) or ketoprofen (0.1 mg/kg) failed to alter the effects of scopolamine. The cue duration titration variant of the 5 choice serial reaction time task is sensitive to disruption by inflammatory abdominal pain in rats, and is reversed by analgesic doses of morphine and ketoprofen. This assay may prove valuable in understanding the basic mechanisms by which pain alters attention processing in rodents, as well as provide a means for assessing the efficacy of novel analgesics for attenuating attention deficits in the presence of pain. Support: NIGMS P01-GM113852.

Disclosures: **T.J. Martin:** None. **T.J. Strassburg:** None. **S.A. Kim:** None. **D.G. Ririe:** None. **J.C. Eisenach:** None.

Poster

524. Pain Models: Behavior

Location: Halls B-H

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Program#/Poster#: 524.10/QQ13

Topic: D.02. Somatosensation: Pain

Title: Assessing age differences in multiple pain behavior assessments

Authors: *C. A. SALCIDO, M. K. GELTMEIER, N. L. MOORE, P. N. FUCHS;
Univ. of Texas At Arlington, Arlington, TX

Abstract: The elderly population is currently the fastest growing demographic of the world. As the prevalence of persistent pain increases with age, the elderly are estimated to comprise a large percent of the 126.1 million American adults that suffer from pain. Yet despite this high prevalence and increasing population, there remains much ambiguity about age-related patterns of pain. This is in part due to equivocal or lack of data from clinical research, driven by patients' reluctance to discuss pain, the misconception that pain is a natural part of the aging process, or age-related cognitive impairment. In pre-clinical models, the overwhelming majority of studies utilize young adult rodents (3-6 month) and there are surprisingly few studies with older animals. Therefore, the purpose of this study was to provide data on a number of preclinical behavioral nociceptive assessments. In this comprehensive assay, one hundred and thirty-seven Sprague Dawley rats ranging from 3-6, 11-15, and 18-24 months were assessed using mechanical paw withdrawal threshold testing (MPWT), thermal threshold testing, formalin, place-escape avoidance paradigm (PEAP), and open-field testing. The results revealed a significant decrease in MPWT assessments for 18-24 month animals compared to 3-6 months. Unexpectedly, there were no significant differences across age for thermal threshold testing. For formalin testing, there were significant increases in mean pain score for 11-15 and 18-24 month animals compared to 3-6 month animals. For PEAP assessments, data revealed significant decrease in avoidance behavior for 18-24 months carrageenan treated animals compared to 3-6 month animals. Lastly, there were significant reductions in the total distance travelled and mean velocity for 11-15 and 18-24 month animals compared to 3-6 month animals. Thus, the results of this study suggest specific patterns of age-related differences, with older animals showing enhanced nociceptive responses involving mechanical reflexive testing and acute inflammatory models, but less responding in a measure of pain affect. Future studies should seek to further assess the relationship between pain and age in order to obtain a comprehensive understanding of pain behaviors with a goal of investigating the underlying biological mechanisms that that influence age related pain-behaviors. Ultimately, such an approach can provide critical information that can translate to clinical populations and lead to improvements in the quality of life of the elderly population.

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Poster

524. Pain Models: Behavior

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Topic: D.02. Somatosensation: Pain

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Conselho Nacional de Desenvolvimento Científico – CNPq

Fundação de Amparo à Pesquisa do Estado de Minas Gerais - Fapemig

Title: Effects of Phα1β on the analgesic and adverse effects of repeated morphine treatment in a mouse model of postoperative pain

Authors: *R. TONELLO[1,2], G. TREVISAN[3], M. V. GOMEZ[4], J. FERREIRA[1];
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Abstract: Background: Opioids are the "gold standard" treatment for postoperative pain, but these drugs also have limiting adverse effects. Thus, adjuvant drugs might be useful in opioid therapy for postoperative pain. An important target for opioid-induced analgesia is the blockade of voltage-gated calcium channels (VGCCs). Phα1β, a peptide purified from the venom of the Brazilian armed spider *Phoneutria nigriventer,* is a VGCC blocker. The aim of the present study was to evaluate effects of Phα1β, on the antinociceptive and adverse effects of morphine in a mouse model of postoperative pain. **Methods:** In this randomized and blinded study, we evaluated the effect of intrathecal (i.t.) injection Phα1β on the antinociceptive (reduction of mechanical hyperalgesia and guarding behavior) and adverse effects (tolerance, constipation, hyperalgesia and withdrawal syndrome) induced through single or repeated (increasing doses, 3 times a day for 3 consecutive days) subcutaneous (s.c.) injections of morphine in C57/BL6 mice subjected to right hind plantar incision. This study was approved by the Committee on the Use and Care of Laboratory Animals of our university (Process No. 117/CEUA/PROPESQ/2013. Procedure PP00872). **Results:** Single injections of Phα1β (100-300 pmol/site, i.t.) or morphine (3-10 mg/kg, s.c.) reversed post-operative nociception in mice. Moreover, in doses that did not produce antinociception when administered separately, Phα1β (30 pmol/site, i.t.) and morphine (1 mg/kg, s.c.) produced an expressive analgesic effect when concomitantly administered. Repeated treatment with morphine in operated mice caused not only tolerance to its antinociceptive effect but also induced paradoxical heat and mechanical hyperalgesia, withdrawal syndrome and constipation. Except constipation, Phα1β (30 pmol/site, i.t.) was able to reverse these adverse effects. **Conclusions:** Taken together, these findings demonstrated that Phα1β not only presented analgesic effects but also potentiated the analgesia and reversed some adverse effects of morphine on operated mice, indicating the potential use of this agent as an adjuvant drug in opioid therapy for postoperative pain.

Disclosures: R. Tonello: None. **G. Trevisan:** None. **M.V. Gomez:** None. **J. Ferreira:** None.

Poster

524. Pain Models: Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 524.12/QQ15

Topic: D.02. Somatosensation: Pain

Support: COBRE Grant P20GM103643

Title: Characterization of pain associated behaviors in a rat model of temporomandibular joint osteoarthritis.

Authors: *S. SANNAJUST[1], I. IMBERT[2], T. KING[2];
[1]Biomed. Sci., [2]Univ. of New England, Biddeford, ME

Abstract: Temporomandibular disorder (TMD) is a musculoskeletal orofacial disorder characterized by pain in the temporomandibular joint (TMJ). Although TMD is a heterogeneous disorder, osteoarthritis (OA) has been found to be present in approximately 15% of the patients. Pain is the primary complaint associated with loss of function in these patients and is a primary motivation for these patients to seek medical care. We characterized a rat model of TMJ OA in which monosodium iodoacetate (MIA) is injected into the TMJ at a dose previously established to produce cartilage loss. We tested the hypothesis that MIA injection into the TMJ produces tactile hypersensitivity, alters meal patterns, and produces ongoing pain using conditioned place preference (CPP). MIA altered meal patterns 7 days post-MIA, with reduced time spent eating and increased the number of meals eaten overnight. Combined, these meal patterns resulted in no changes in overnight time spent eating compared to saline control rats. In addition, no weight loss was observed in MIA treated rats compared to saline controls. These changes in meal eating patterns were not observed D14 post-MIA, with no change in meal duration or number of meals eaten overnight compared to saline treated controls. These data indicate that MIA injection into the TMJ produced a transient change in meal eating behaviors that resolved by 14 days post-MIA. MIA into the TMJ produced tactile hypersensitivity at the joint and infraorbital test sites within 7 days that continued through 14 days post-injection. Administration of lidocaine systemic lidocaine (10 mg/kg, s.c.) D14 post-MIA reversed the MIA-induced tactile hypersensitivity within 60 min post-injection. In addition, MIA treated rats demonstrated CPP to systemic lidocaine (10 mg/kg, s.c.) indicating that this dose of lidocaine alleviates ongoing pain. These observations indicate that MIA-induced OA of the TMJ produces tactile hypersensitivity and ongoing pain that can be reversed with lidocaine 14 days post-MIA. Notably, these pain behaviors were observed at a time when meal eating behaviors had normalized, indicating that the rats adapted to the ongoing joint pain. These results indicate that we have developed a model of osteoarthritis of the TMJ. This model may lead to improved mechanistic understanding of the mechanisms driving TMJ pain that is necessary for development of improved therapeutic

strategies for this subpopulation of TMD patients. This work was funded in part by COBRE grant P20GM103643.

Disclosures: **S. Sannajust:** None. **I. Imbert:** None. **T. King:** None.

Poster

524. Pain Models: Behavior

Location: Halls B-H

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Program#/Poster#: 524.13/QQ16

Topic: D.02. Somatosensation: Pain

Title: Effect of pregabalin on spinal wide dynamic range neuronal activity, burrowing behavior of reserpine-induced fibromyalgia in rats

Authors: V. GOURA, A. VUYYURU, R. KALLEPALLI, P. JAYARAJAN, R. NIROGI, *S. M. IRAPPANAVAR;
SUVEN LIFE SCIENCES LTD, HYDERABAD, India

Abstract: Chronic pain is an abnormal and non-protective response which represents a major health problem. Chronic widespread pain is a core symptom in fibromyalgia patients. We investigated the effect of pregabalin in central sensitivity syndrome (like fibromyalgia). Fibromyalgia was induced in rats using reserpine (1 mg/kg s.c., once daily for three consecutive days), basal reading was obtained on day 4 after final dose of reserpine using Von Frey filaments / analgesymeter. Selected rats were used for experimentation. Central sensitization was investigated using in-vivo electrophysiology in dorsal horn neurons of fibromyalgia in rats. Central sensitization clinically and physiologically represents allodynia and hyperalgesia conditions, we investigated for the same using Von Frey monofilaments (allodynia) and analgesymeter (hyperalgesia). Further we investigated to translate “bedside-to-bench” outcomes from the human pain phenotype to rodents for the first time in reserpine induced fibromyalgia in rats through burrowing behavior (which evaluates non-evoked and unbiased pain response). Results indicate pregabalin significantly reversed electrically evoked response of the dorsal horn neurons, attenuated mechanically evoked pain response using Von Frey monofilaments and analgesymeter. Analgesic activity of pregabalin significantly reversed burrowing defects due to pain in rodents. Results suggest that pregabalin may be effective against multiple aspects of fibromyalgia.

Disclosures: **V. Goura:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **A. Vuyyuru:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **R. Kallepalli:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **P. Jayarajan:**

A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **R. Nirogi:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD. **S.M. Irappanavar:** A. Employment/Salary (full or part-time): Suven Life Sciences LTD.

Poster

524. Pain Models: Behavior

Location: Halls B-H

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Topic: D.02. Somatosensation: Pain

Support: Baptist Health Foundation

Nathan Shock Center of Excellence

AG013319

AG047514

Title: The effect of aging on peripheral kappa opioid receptor-mediated antinociceptive signaling

Authors: *E. JENNINGS, H. R. SMITH, P. LOCOCO, J. ZAMORA, T. CHAVERA, K. BERG, W. CLARKE;
Pharmacol., Univ. Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Pain management in the growing elderly population is a major medical challenge. Targeting peripheral opioid receptors could provide effective and safer medications for the elderly, bypassing centrally mediated adverse effects. However, little is known regarding the effects of aging on nociceptor (pain-sensing neuron) responsiveness or the functionality of opioid receptors expressed on nociceptors. Here, we determined the effects of the kappa opioid receptor (KOR) agonist, Salvinorin A (Sal A), on nociceptors in young (4-months-old) and aged (26-months-old) Fisher x Brown Norway rats. Behavioral responses to noxious heat were measured following intraplantar injections of opioid agonists. Intraplantar (i.pl.) administration of Sal A produced significant antinociceptive responses in aged rats. By contrast, antinociceptive responses were not observed in young rats at this dose. Similarly, KOR-mediated inhibition of adenylyl cyclase activity was greater in nociceptors derived from aged versus young rats. Intraplantar injection of young rats with Sal A produced significant antinociceptive responses following exogenous administration (i.pl.) of arachidonic acid (AA). Administration of AA had no additive effect on antinociceptive responses in aged rats. Our results indicate that aging alters kappa opioid receptor sensitivity to agonists, such that in non-inflamed tissue Sal A produces an

antinociceptive effect in aged rats only. We propose that peripherally-restricted kappa agonists may be a valuable analgesic strategy for treating pain in the elderly.

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Poster

524. Pain Models: Behavior

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Topic: D.02. Somatosensation: Pain

Support: KAKENHI Grant 15H04968

Title: Confirmation of pain-related behavior changes of newly developed persistent post-operative pain model by using MyD88 and TRIF knock out mice

Authors: *A. NAKAE[1], K. NAKAI[2], Y. KUMAGAI[1], K. HOSOKAWA[3], Y. YOSHIOKA[1]; [1]Osaka Univ. Immunol. Frontier Res. Cent, Suita, Japan; [2]Univ. of Fukui hospital, Eiheiji, Japan; [3]Osaka Univ. Grad. Sch. of Med., Suita, Japan

Abstract: Background: Persistent post-operative pain has recently been recognized as adversely affecting the patients' quality of life. The International Association of the Study of Pain defines persistent postoperative pain (PPP) as pain that persists for more than 3 months after surgery. However, general animal models of postoperative pain show that their pain lasts a maximum of two weeks. Whereas, based on a chronic constriction injury model, the nerve injury impedes sensory functions of the affected area for about two weeks. Thus, there is a need to develop a new animal model of PPP. This model can be used to evaluate their condition continuously from just after surgery to chronic pain periods. Inflammatory response is crucial when the symptoms of pain appears after surgery. It is well known that the MyD88 and TRIF pathway is important for innate immune reaction. A few studies revealed that these genes' knock out mice showed less pain-related behavior by modulating the cytokine dependent pain pathway. The aim of this study is to confirm the significant changes of pain-related behavior of the newly developed PPP model by using MyD88-/- and TRIF-/- mice. Methods: All experiment protocols in this study were approved by the Animal Care and Use Committee of Osaka University. C57Bl/6J mice, MyD88-/- mice and TRIF-/- mice were anesthetized and laid in the prone position. The new PPP model was developed accordingly as follows; the incision is made around plantar skin and the flap is raised with preserving posterior tibial vessels and nerves. Afterwards, the flap is sutured into the original position. Behavioral tests were carried out before the surgery and after the surgery-1 to 3

days post-surgery and 1, 2 and 4 weeks post-surgery. Von Frey Filaments were delivered to the injured hind paw. For behavioral experiments, data were evaluated by two-way analysis of variances followed by a Tukey-Kramer multiple comparison test. Results below 0.05 is considered significant. Results & Discussion: The PPP surgery to the hindpaw induced mechanical hyperalgesia that startedThank 1 day after surgery and lasted 4 weeks after surgery. Compared to the wild type mice, MyD88$^{-/-}$ and TRIF$^{-/-}$ mice showed significantly higher withdrawal threshold at 1 and 2 days after surgery, whereas, 2 and 4 weeks after surgery, MyD88$^{-/-}$ showed higher withdrawal threshold than wild type mice. These results showed that the new PPP model could be used for the study of PPP in mice and MyD88$^{-/-}$ and TRIF$^{-/-}$ may be important factors for maintaining persistent pain states.

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Poster

524. Pain Models: Behavior

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant R25 GM 59994-09

NIH Grant SC1NS078778

Title: Activation of spinal membrane estrogen receptors rapidly attenuates opioid receptor-like 1 (ORL1) receptor-mediated modulation of nerve injury-induced tactile hypersensitivity in the rat

Authors: *D. M. HECKARD[1], S. NAG[2], S. S. MOKHA[2];
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Abstract: Numerous studies have reported that women have a higher prevalence of chronic pain disorders such as temporomandibular joint disorder, fibromyalgia etc. We have previously shown that estrogen attenuates opioid receptor like -1 (ORL1) receptor mediated antinociception in females [Claiborne et al. J Neurosci. 26:13048-53, 2006]; and down regulates the ORL1 gene expression [Flores et al. Neurosci. 118:769-78, 2003]. Recently, we have shown that activation of selected membrane estrogen receptors (mERs: GPR30, Gq-MER, ERα, but not ERβ) abolishes ORL1-mediated acute thermal antinociception via an ERK2-dependent non-genomic mechanism [Small et al. Neurosci. 255:177-190, 2013]. However, whether the activation of mERs attenuates ORL1-mediated modulation of nerve injury-induced tactile hypersensitivity is unknown. Thus,

the present study was designed to investigate whether activation of spinal mERs attenuates ORL1-mediated modulation of nerve injury-induced mechanical hypersensitivity. We employed the spared nerve injury (SNI) model as previously described by Decosterd and Woolf [Pain. 87:149-58, 2000] to induce mechanical hypersensitivity in male and ovariectomized (OVX) female Sprague Dawley rats. Stretched PE10 cannulae were also implanted intrathecally at the same time. After a 7-day recovery period, E2BSA, a membrane impermeable analog of estradiol, or a selective mER agonist and OFQ, the endogenous ligand for the ORL1, were co-administered intrathecally. Paw withdrawal thresholds (PWTs) were recorded using an automated dynamic plantar aesthesiometer. SNI significantly reduced PWTs in both males and OVX females. Intrathecal administration of OFQ significantly increased PWTs in both groups with or without SNI. E2BSA as well as selective mER activation abolished OFQ-induced increase in PWTs. Thus, we conclude that activation of mERs rapidly attenuates ORL1-mediated modulation of nerve injury-induced mechanical hypersensitivity. This may constitute a biological mechanism that increases female vulnerability to the development of chronic pain disorders.

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Poster

524. Pain Models: Behavior

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Topic: D.02. Somatosensation: Pain

Support: Grant-in-Aid (No.26462726) from the Ministry of Education, Culture, Sports, Science and Technology, Japan

Title: The role of spinal adrenoceptor subtypes in trigeminal nerve injury-induced mechanical hypersensitivity of rats

Authors: *K. NAKAI[1], A. NAKAE[2], T. KUBO[3], Y. MINEGISHI[1], Y. FUJINO[4], K. HOSOKAWA[3];
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Abstract: (Background) Substantial evidences revealed that descending spinal adrenergic transmission had an important role in the process of pain modulation. Spinal administration of the alpha 1 and alpha 2 adrenergic receptor agonists has been shown to produce antinociception. The contribution of spinal alpha 1and alpha 2 adrenergic receptor subtypes in inhibiting the

trigeminal neuropathic pain remained unclear. Chronic constriction injury to the infraorbital nerve (ION-CCI) has been proven a useful model for trigeminal neuropathic pain. The present study evaluated the possible role of spinal alpha1A, alpha2A, and alpha2C adrenergic receptors in ION-CCI rat model.
(Material and Methods) Male Sprague Dawley rats underwent unilateral CCI to the right ION. Two nylon (5-0) ligatures were tied around the ION. Series of von Frey filaments were used to determine pain hypersensitivity to mechanical stimulation on day 14 after surgery. A polyethylene (PE-10) catheter was implanted for upper cervical spinal injection of drugs. The rats were allowed to recover for 7 days. The time course of the antiallodynic effects and the dose-response effects of intrathecally administered an alpha 1A adrenergic receptor agonist A 61603, an alpha 1A adrenergic receptor antagonist WB 4101, an alpha 2A adrenergic receptor agonist guanfacine, an alpha 2A adrenergic receptor antagonist BRL 44408, an alpha 2C adrenergic receptor agonist nitrobiphenyline, and an alpha 2C adrenergic receptor antagonist JP 1302 were examined. The time course data for the dose-response effects were analyzed by two-way analysis of variance and Tukey-Kramer multiple-comparison test. (Results) Intrathecal administration of A 61603, guanfacine, and nitrobiphenyline increased mechanical thresholds in a dose dependent manner (P <0.05). Intrathecal administration of WB 4101, BRL 44408, and JP 1302 did not alter mechanical thresholds. (Conclusions) The results indicated that descending pain inhibitory system in the spinal cord may be activated via spinal alpha1A, alpha2A, and alpha2C adrenergic receptors in a rat model of trigeminal neuropathic pain.

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Poster

524. Pain Models: Behavior

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Topic: D.02. Somatosensation: Pain

Support: Mexican Academy of Sciences

Title: Oxytocin-induced analgesia is facilitated by the vasopressin 1b receptor in mice

Authors: *J. C. MORALES-MEDINA[1], H. K. CALDWELL, 44240[2];
[1]Ctr. for Res. and Advanced Studies, Tlaxcala, Mexico; [2]Biol. Sci., Kent State Univ., kent, OH

Abstract: The neuropeptides arginine vasopressin (Avp) and oxytocin (Oxt) act as strong antinociceptive agents. However, the receptors that mediate the antinociceptive effects of Avp

and Oxt remain unknown. While the physiological effects of Avp are mediated by three receptors Avp 1a receptor (Avpr1a), Avp 1b receptor (Avpr1b), Avp 2 receptor (V2); the effects of Oxt are modulated a single receptor subtype, the Oxt receptor (Oxtr). However, all four receptors are sensitive to both nonapeptides. In the present study, we aimed to investigate whether genetic deletion of the *Avpr1b* (Avpr1b KO), in mice, had antinociceptive effects on their response to Avp, Oxt and osmotic stress (OS) challenge in the absence of injury and after peripheral inflammation with the complete Freund's adjuvant (CFA). Avpr1b KO mice had increased mechanical thresholds after Oxt administration in control and CFA-treated mice. Most notably, Avpr1b KO mice had reduced mechanical hypersensitivity after CFA administration, with no changes in paw edema. Thus, these results suggest Avpr1b is involved in nociception and facilitates the antinociceptive effects of Oxt.

Disclosures: J.C. Morales-Medina: None. **H.K. Caldwell:** None.

Poster

524. Pain Models: Behavior

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Topic: D.02. Somatosensation: Pain

Support: Augusta University College of Science and Mathematics

Augusta University Center for Undergraduate Research and Scholarship

Augusta University Office of Faculty Development and Teaching Excellence

Augusta University Department of Psychological Sciences

Title: Effects of monoamine uptake inhibitors on pain-depressed behavior in mice.

Authors: K. ALEXANDER, T. RODRIGUEZ, A. SARFO, T. B. PATTON, *L. L. MILLER; Psychological Sci., Augusta Univ., Augusta, GA

Abstract: Pain-related decreases in behavior are among the primary diagnostic and treatment concerns for physicians, but preclinical laboratory research on pain often does not address this important endpoint. For instance, intraperitoneal injection of dilute lactic acid is a nociceptive pain stimulus that stimulates a stretching response in rodents, and drug-induced decreases in acid-stimulated stretching is interpreted as a pain-relieving effect. Such pain-related stimulation of behavior bears little resemblance to clinically-relevant pain-related depression of behavior. Studies that address the motivational consequences of pain may improve understanding of pain

mechanisms, and contribute to the development of new pain treatments. In the present study, we modeled pain-related depression of behavior by examining nesting behavior in male ICR mice. Nest building is an innate mouse behavior that occurs at a high rate under control conditions. Nesting is depressed by intraperitoneal injection of dilute lactic acid (0.56%), and the clinically effective nonsteroidal anti-inflammatory drug (NSAID) ketoprofen blocks acid-induced depression of nesting. Monoamine uptake inhibitors have a history of use as antidepressants, but have more recently been used in the treatment of pain. This project examines effects of monoamine uptake inhibitors with varying selectivity for serotonin (5HT), norepinephrine (NE) and dopamine (DA) transporters on pain-related depression of nesting. Citalopram (5HT-selective), nisoxetine (NE-selective), milnacipran (mixed action, 5HT/NE-selective), and bupropion (DA-selective) were evaluated for their ability to block lactic acid-induced depression of nesting and lactic acid-stimulated stretching behavior. Consistent with previous findings in other assays of acute pain-related depression of behavior, monoamine uptake inhibitors lacking significant activity at dopamine transporters do not block acid-depressed nesting, but do block acid-stimulated stretching. These findings suggest that serotonergic and noradrenergic monoamine uptake inhibitors have limited efficacy in the treatment of acute pain-related depression of behavior. Unlike findings from studies using other models of acute pain-related depression of behavior, the DA-selective uptake inhibitor bupropion also did not block acid-depressed nesting. Together these findings support the use of assays of pain-depressed behavior to study motivational aspects of pain, but point to the need for more work to identify determinants of the expression, mechanisms, and treatment of pain-related depression of behavior.

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Poster

524. Pain Models: Behavior

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Program#/Poster#: 524.20/RR3

Topic: D.02. Somatosensation: Pain

Support: CDMRP MR157005

Title: Sound stress activates corticotropin-releasing factor receptor-2 (CRFR2) in the lumbar spinal cord of rats with thermal injury

Authors: *N. SOSANYA, A. TREVINO, R. CHAVEZ, R. CHRISTY, B. CHEPPUDIRA; Battlefield Pain Mgmt., Inst. Of Surgical Res., JBSA Ft Sam Houston, TX

Abstract: Introduction: Studies have shown that exposure to stressful stimuli alters basal pain processing and also exacerbates injury induced pain. However, the underlying mechanism associating stress and pain remains unclear. Corticotropin-releasing factor (CRF) signaling pathways play an important role in stress-induced disorders including pain. The present study examined the involvement of CRFR2 in sound stress-induced changes in hyperalgesic behaviors of rats with thermal injury.
Methods: Sound stress in male Sprague Dawley rats was induced by exposing them to 105 dB tone over several different frequencies ranging from 11 to 19 kHz, each lasting for 5- 10 s within a 30 min period, over 3 consecutive days. One day after induction of sound stress, rats were deeply anesthetized and subjected to unilateral partial-thickness thermal injury by placing a pre-heated (100° C) soldering tip on the mid-plantar surface of the right hind paw for 30 sec. The development of hyperalgesic behavior was assessed at 1, 4, 7, and 14 days using the Hargreaves' thermal test. Following behavioral experiments, the animals were euthanized and the lumbar spinal cord section was harvested and analyzed for CRF2 receptors using qRT-PCR and gel electrophoresis validation of PCR products.
Results: Consistent with earlier reports, the thermal injury alone led to persistent thermal hyperalgesia on the injured paw (ipsilateral), but not on the contralateral paw. However, thermal injured rats that had prior exposure to sound stress exhibited greater magnitude of hypersensitivity compared to control rats (non-stressed + burn injured rats). Additionally, the mRNA expression level of CRFR2 was significantly upregulated in the lumbar spinal cord of thermal injured rats that had prior exposure to sound stress, but not in rats that just received thermal injury.
Conclusion: These novel results indicate that sound stress alters CRF2 receptors expression in the lumbar spinal cord. This data suggests that sound stress following thermal injury activates and releases CRF which in turn binds CRF2 receptors resulting in exacerbated pain behaviors.

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Poster

524. Pain Models: Behavior

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Support: UT Arlington Psychology Bridging funds

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Title: Conditioned place preference/aversion facilitates prolongation of migraine pain-related affective behavior after single meningeal CFA administration

Authors: *S. KOKANE[1], C. NAIG[2], A. NGO[2], F. TAO[3], Q. LIN[1];
[1]Psychology, [2]Univ. of Texas At Arlington, Arlington, TX; [3]Biomed. Sci., Texas A&M Hlth. Sci. Ctr. Baylor Col. of Dent., Dallas, TX

Abstract: Current animal models of chronic headache (migraine) involve activating nociceptors of the trigeminal afferents present on the meninges by repeatedly administering a mixture of inflammatory mediators in to the cisterna magna or via direct topical application on the dura. Single administration of inflammatory mediators has been shown to induce acute migraine-like headache that lasts for up to 24-48 hours.In our study, we injected 5 µl of Complete Freund's adjuvant (CFA) directly on to the dura of adult C57BL/6 male mice. Von Frey filaments were used to determine cutaneous allodynia in the craniofacial region. We also used the conditioned place preference/aversion (CPP/A) paradigm to determine the persistence of negative affective component associated with migraine-like headache.We observed that cutaneous allodynia persisted for 72 hours after single CFA application. After 72 hours, mechanical thresholds in the craniofacial regions reverted back to baseline. Interestingly however, after conditioning the animals using the CPP/A paradigm, we observed that the animals demonstrated a prolonged preference to the chamber that was paired with "no pain" condition for up to 8 days after single CFA application to the dura.These results indicate that although physiological effects of single CFA application persisted for acute stages, the negative affective component associated with migraine pain persisted for much longer which was potentially facilitated by CPP/A paradigm. Thus, our pilot study demonstrates a potential use of CCP/A paradigm in the possible development of a chronic migraine headache model.

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Poster

524. Pain Models: Behavior

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Topic: D.02. Somatosensation: Pain

Support: NIH/NINDS R01NS086830

Title: Calcitonin gene-related peptide mediated development of headache-related behaviors in a clinically relevant rat model of post-traumatic headache

Authors: *D. BREE[1], D. LEVY[2];
[1]Headache Res. Lab., Boston, MA; [2]Anesthesia, Beth Israel Deaconess Med. Center, Harvard Med. Sch., Boston, MA

Abstract: One of the most common, debilitating and difficult symptoms to manage after a concussion, or mild traumatic brain injury (mTBI) is chronic posttraumatic headache (PTH) with migraineous features. The mechanisms that mediate the onset and maintenance of PTH remain elusive in part due to the lack of an animal model that mimics the most common type of head trauma and exhibits pain behaviours similar to those seen in individuals with PTH. The present study aimed to characterize for the first time headache-related behaviours in a clinically relevant rat model of mTBI, which is the major type of trauma associated with PTH. Under isoflurane anaesthesia, mTBI was induced in adult male Sprague-Dawley rats by a closed head injury using a modified Marmotou weight-drop device. Post-concussion behavioural characterisation involved assessment of cephalic mechanical pain hypersensitivity, using calibrated von Frey monofilaments and ongoing pain, using the conditioned place preference paradigm. Injured rats displayed increased cephalic mechanical hypersensitivity up to 7 days post-injury. Acute administration of the anti-migraine drug Sumatriptan (1mg/kg i.p.) attenuated the mechanical hypersensitivity and produced conditioned place preference at discrete time points (Day 7) following head injury. Following resolution of the cephalic hypersensitivity by Day 14 post-injury, administration of the common migraine trigger glycerol trinitrate (GTN) (100µg/kg i.p.) resulted in a renewed pain-related behavioural phenotype in mTBI but not in sham animals, which was aborted by acute treatment with sumatriptan. Targeting CGRP, using weekly administration of a blocking mAb (10mg/kg i.p.), starting immediately after the head injury, inhibited the acute development of cephalic mechanical hypersensitivity as well as that triggered by GTN later on. The current data suggest that mTBI in rats is associated with the emergence of a headache-related behavioural phenotype, which is amenable to treatment with common and novel putative migraine therapies and may serve as viable model for the study of the pathophysiology of post-traumatic headache.

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Poster

524. Pain Models: Behavior

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Support: NRF Grant 2014R1A1A2054819

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Title: Electroacupuncture attenuates inflammatory pain in the rat model of CFA-induced arthritis: involvement of peripheral resolvin

Authors: Y. KIM[1], B. JI[1], C. LI[1], J. LEE[1], *S. KOO[1,2];
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Abstract: Resolution of acute inflammation is known to be biochemical processes that enable inflamed tissues to return to homeostasis. Resolvin, a mediator of resolving inflammation, is known to reduce inflammation and also attenuate inflammatory pain. Acupuncture is widely used for various painful conditions include knee arthritis. In the present study, we investigated the involvement of peripheral resolvin in the analgesic effects of electroacupuncture (EA) in the rat model of inflammatory pain.
Arthritis was induced by injecting 125μl CFA into the knee joint cavity of the right hind limb. To evaluate the level of pain, the stepping pressure of the hind limb (weight bearing force; WBF) was measured before and after arthritis induction. The rats were divided into 3 groups (anesthesia control and two EA treated groups). For the EA treatment, a pair of needles was inserted in ST36 or SP6 point in a depth of 5 mm. EA treatment was delivered under gaseous anesthesia for 30 min via parameter of 10 Hz of frequency and 1 mA of intensity. The animals in the control group were given anesthesia only for same period of EA treatment. After measuring the daily baseline, EA was given from the post injection day (PID) 2 once a day for 12 times. For the resolvin analysis, we collected synovial fluid of the right knee on the PID 2, 3, and 7 and measured the amount of resolvins and inflammatory cytokines using multiplex ELISA.
The WBF of affected limb was rapidly decreased from the PID 1 presumably due to the inflammatory pain. The pain behavior was maximized on PID 2 and significantly lowered for all period of experiment in control group. However, the WBF of ST36 group was increased significantly compare to those of the SP6 or control group. Furthermore, the WBF of ST36 group at PID 7 was reached at those of control group at PID 14 meaning repeated EA treatment of ST36 made early recovery of inflammatory pain. In the ELISA analysis, RvD1 was increased significantly in the synovial fluid of ST36 treated animals compare to amount of SP6 or control group at PID 2.

Data suggest that EA applied to ST36 point shows analgesic and pro-resolving effects in the rat model of arthritis in a point specific manner. Peripheral RvD1 is probably involved in the underlying mechanism of EA effects in the inflammatory pain. Therefore, it cloud be concluded that EA can not only reduce inflammatory pain but also resolve the inflammation.

Disclosures: **Y. Kim:** None. **B. Ji:** None. **C. Li:** None. **J. Lee:** None. **S. Koo:** None.

Poster

524. Pain Models: Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 524.24/RR7

Topic: D.01. Sensory Disorders

Support: VA Grant IK2 RX-002010-01

Title: Investigation of neuronal circuits underlying light-aversive behavior

Authors: ***L. P. SOWERS**[1,2], B. J. REA[1,2], R. TAUGHER[1,2], Y. KIM[1], A. KUBURAS[1], L. TERAN[1], S. MA[1], J. WEMMIE[1,2], A. RUSSO[1,2];
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Abstract: Veterans returning from active duty are at an increased risk for post-traumatic headache (PTH) and migraine. Migraine alone affects 10% of men and 25% of women, with the lifetime risk increasing to 18% and 43% respectively. Sensory abnormalities are present in individuals with PTH and migraine including extreme light and sound sensitivity. Light sensitivity in patients with PTH or migraine can be debilitating and treatments are lacking. One reason interventions and treatments continue to fall short is that there is a poor understanding of the relevant neuroanatomical correlates that underlie sensory changes in headache. Calcitonin gene-related peptide (CGRP) is a critical neuropeptide involved in pain signaling and has recently come to the forefront of migraine research where it contributes to headache and associated sensory abnormalities. In this study we attempt to identify anatomical regions where CGRP could act to induce light-aversive behavior in migraine and PTH. The posterior thalamus (Po) has been suggested to be a brain region that could integrate light and pain. In addition to the Po, the periaqueductal grey (PAG) has been suggested to play a role in migraine headaches. We hypothesized that CGRP acts as a neuromodulator in the Po and/or the PAG to induce light aversive behavior. To test this hypothesis, we used two targeted approaches to probe areas of the brain thought to be important in the development of photophobia. The first was direct calcitonin gene related peptide (CGRP) injection into the Po. The second was optogenetic stimulation using channelrhodopsin driven by the CaMKII promoter to stimulate either the Po or the PAG. We

found that CGRP injection in the Po as well as optical stimulation of the Po induces significant light aversive behavior, without increased anxiety in light-independent assays. However, in contrast to the Po, PAG stimulation led to both light aversion and light-independent anxiogenic behavior. These data suggest that the Po can induce light aversion associated with CGRP actions, while the PAG may trigger not only the Po, but also other brain regions involved in anxiogenic behaviors. These results may begin to shed light on the complex circuitry of light-aversive behaviors in mice.

Disclosures: L.P. Sowers: None. **B.J. Rea:** None. **R. Taugher:** None. **Y. Kim:** None. **A. Kuburas:** None. **L. Teran:** None. **S. Ma:** None. **J. Wemmie:** None. **A. Russo:** None.

Poster

524. Pain Models: Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 524.25/RR8

Topic: D.02. Somatosensation: Pain

Title: A crosstalk between neuropathic pain and depression establishing a novel drug development platform

Authors: *S. EDUT[1], G. RICHTER-LEVIN[2];
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Abstract: Pain, especially chronic pain, is an emotional condition as well as a physical sensation. It is a complex experience that affects thought, mood, and behavior. Clinical reports estimate that over half of chronic pain patients also possess a robust stress-like component, reflecting a significant affective cognitive aspect. The physiologic basis for the comorbidity of chronic pain and depressive illnesses is not well understood, impeding appropriate therapeutic approaches from being adopted. Studies focusing on mood disorders have indicated that stress affects different forms of synaptic plasticity and memory processes in the hippocampus. Experience of continued stress can impair high-frequency stimulation (HFS) to induced long-term potentiation (LTP). Stress-induced modifications in synaptic plasticity could therefore represent a useful model to examine whether physical chronic pain state affects subsequent responsiveness to psychogenic stressors. We have recently described a relatively unique role for the dentate gyrus (DG) of the hippocampus. Under stressful conditions, the DG response is variable and complex, much like the behavioral outcomes of such circumstances. In this study, we investigated the impact of chronic pain on activity and plasticity in the DG. We have employed the Sciatic nerve ligation (SNL) model of neuropathic pain, a most common form of chronic pain and have examined synaptic transmission/plasticity in the DG of anaesthetized rat.

Behavioural tests such as novel place exploration and elevated plus maze were also conducted. We display that activity and plasticity in the DG are affected by exposure to stress, but in a complex way. Local circuit activity is sensitive to the different kind of stress and can be a measure to differentiate between effects of acute and chronic pain. Freezing in a novel place was also found to be a group depended behaviour. Till now we learned that both chronic stress (NP) and acute stress (SHAM) affect the LTP in the DG

Disclosures: S. Edut: None. **G. Richter-Levin:** None.

Poster

524. Pain Models: Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 524.26/RR9

Topic: G.03. Emotion

Support: Subcontract Delivra Inc.

InterVivo Solutions

Title: Validation of a translational pain questionnaire assessing behavioral quality of life measures in a naturalistic canine model of osteoarthritic pain and urate-induced pain

Authors: *J. A. ARAUJO[1], S. KELLY[2], J. BAULK[2], D. ARAUJO[2], C. DE RIVERA[2], D. BARONOWSKI[3], J. GABRIELE[3];
[1]InterVivo Solutions, Toronto, ON, Canada; [2]Vivocore Inc., Toronto, ON, Canada; [3]Delivra Inc, Burlington, ON, Canada

Abstract: Evaluation of pain in animal models typically relies on responsive measures, such as withdrawal reflexes from noxious stimuli. Because affective pain measures relevant to quality of life often are not included or not available, the translational value of animal models of pain is limited. In the current study, a questionnaire assessing pain during performance of standard daily functions was validated using approved NSAID pain therapeutics in both naturalistic and induced canine models of pain. Specifically, the naturalistic canine model employed aged dogs with radiographic evidence of osteoarthritis scored by a veterinary radiologist and the induced model employed injection of sodium urate into the stifle of normal dogs. Both models were used to evaluate the utility of the pain questionnaire under control conditions compared to treatment with meloxicam, which is approved for human and veterinary use. For both models, the questionnaire evaluated functional ability and observable pain of dogs performing standard behaviors such as walking, trotting, galloping, stepping over obstacles, climbing and descending

stairs, rearing for food and jumping down from a perch. In aged dogs with naturally occurring osteoarthritis, treatment with meloxicam (0.1 mg/kg PO and 0.4 mg/kg SC) reduced measures of pain compared to negative control and also when compared to the first day of treatment. Moreover, injection of sodium urate into the stifle joint produced a significant increase in pain evident 4, 8 and 24 hours after injection. Treatment with meloxicam (0.2 mg/kg PO) significantly decreased pain measures at all the same time-points. This demonstrates that meloxicam, administered according to the veterinary approved labeling reduces pain measured by the quality of life based questionnaire used in the current study. Therefore, the improvement in pain observed under meloxicam validates the pain questionnaire used in the current study. Given meloxicam improves measures of pain in both dogs and humans, the pain questionnaire may provide a translational assessment of affective pain that is clinically translational. Additional studies are required to extend the utility of the current model beyond effects of NSAIDS.

Disclosures: J.A. Araujo: A. Employment/Salary (full or part-time): InterVivo Solutions. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Delivra Inc. **S. Kelly:** A. Employment/Salary (full or part-time): Vivocore Inc. **J. Baulk:** A. Employment/Salary (full or part-time): Vivocore Inc. **D. Araujo:** A. Employment/Salary (full or part-time): Vivocore Inc. **C. de Rivera:** A. Employment/Salary (full or part-time): Vivocore Inc. **D. Baronowski:** A. Employment/Salary (full or part-time): Delivra Inc. **J. Gabriele:** A. Employment/Salary (full or part-time): Delivra Inc..

Poster

525. Pain Models: Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 525.01/RR10

Topic: D.02. Somatosensation: Pain

Support: Veterans Affairs A NURA-009-13S

Title: Involvement of anandamide in differential sympatho-sensory control

Authors: *C. DEAN-BERNHOFT[1], C. J. ROBERTS[2], F. A. HOPP[2], Q. H. HOGAN[2]; [1]Anesthesiol., Med. Col. of Wisconsin, Zablocki VA Med. Ct, Milwaukee, WI; [2]Med. Col. of Wisconsin and Zablocki VA Med. Ctr., Milwaukee, WI

Abstract: Sympatho-sensory integration in the dorsal periaqueductal gray (dPAG) is fundamental to the response to acute stress. A differential increase in sympathetic nerve activity and blood pressure accompany decreased sensitivity to pain (antinociception) to allow escape from a stressor. This study was undertaken to address disassociation of the two components, which could provide therapeutic potential to decrease the response to pain without the unwanted side effects of increased sympathetic outflow and blood pressure. Blood pressure, whole renal sympathetic nerve activity (RSNA) and unit discharges of dorsal horn neurons (DHN) responding to high intensity mechanical stimulation of the hindpaw were recorded from anesthetized rats. The synaptic excitant D,L-homocysteic acid (30 nl, 4 mM) or the endocannabinoid anandamide (AEA) (30 nl, 50 μM) were microinjected into the dPAG, targeting coordinates from which a stress response can be evoked. Microinjections were made at 0.5 mm depth intervals and blue dye microinjected at the deepest microinjection site for subsequent histological mapping. Electrophysiological data was analyzed using Spike2 software and Sigma Plot 11. DLH and AEA could evoke (i) a differential pattern of neural response consisting of increased RSNA with decreased DHN or (ii) a decrease in DHN without change in RSNA. dPAG sites were also found from which no sympathy-sensory responses were elicited. These data demonstrate that there is a role for cannabinoids in the integration of sympathoexcitation and sensory inhibition in the dPAG. Further, selective inhibition of DHN activity by dPAG neurons could provide a cannabinergic target with therapeutic potential for analgesia. Supported by Veterans Affairs A NURA-009-13S

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Poster

525. Pain Models: Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 525.02/RR11

Topic: D.02. Somatosensation: Pain

Support: American Heart Association (AHA) 44081 (M.J.P)

NIH/NIGMS RO1 GM112747 (A.N.A)

School of Dentistry URC pilot research project (A.N.A)

Title: Female specific regulation of postoperative pain by sensory neuronal prolactin receptor and extrapituitary prolactin

Authors: *M. J. PATIL[1], V. GOFFIN[2], M. A. HENRY[3], A. N. AKOPIAN[1];
[1]Endodontics, UT Hlth. Sci. Ctr., San Antonio, TX; [2]Inserm and Univ. Paris Descartes, Paris, France; [3]Univ. of Colorado, Denver, CO

Abstract: Aim of Investigation: Sex-dependent regulation of the nociceptive processing is estrogen and/or testosterone dependent, but detailed mechanisms are largely unknown. We have previously shown that prolactin (PRL) levels in serum and extra-pituitary tissues are dually regulated by estrogen and tissue injury. Such properties of the prolactin system (PRL and PRL receptor - Prlr) imply that it could play a critical role in sex-dependent regulation of pain. Accordingly, we investigated whether the prolactin system is involved in sex-dependent regulation of pain and the cellular mechanisms that are responsible for such regulation. **Methods:** Classical hindpaw incision on mice and rats was used as an acute postoperative pain model. Hypophysectomised male and female rats were used to evaluate the contribution of extra-pituitary PRL in mediating postoperative hypersensitivity. We immunohistochemically (IHC) characterized the expression of PRL in various cell types at surgical site and spinal cord. Nav1.8-cre/Prlr-lox conditional knockout (CKO) was used to evaluate the role of sensory neuronal Prlr in postoperative pain. **Results:** Ablation of Prlr in sensory neurons caused significant reduction in postoperative thermal and mechanical hypersensitivity exclusively in females. Hypophysectomy in male and female rats did not cause significant change in both postoperative thermal and mechanical hypersensitivity in females. PRL is expressed in sensory neuronal peripheral and central terminals as well as astrocytes. The role of PRL in astrocytes in female-specific postoperative pain was examined. **Conclusions:** Sensory neuronal Prlr controls sex-dependent regulation of postoperative hypersensitivity only in females. Extra-pituitary PRL released locally at the site of incision and/or spinal cord, but not the PRL originated from the pituitary, mediates sex-dependent regulation of postoperative hypersensitivity via autocrine and paracrine mechanisms.

Disclosures: M.J. Patil: None. **V. Goffin:** None. **M.A. Henry:** None. **A.N. Akopian:** None.

Poster

525. Pain Models: Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 525.03/RR12

Topic: D.02. Somatosensation: Pain

Support: Funding from the Intramural Research Program of the NIH's National Center for Complementary and Integrative Health

Title: Fear of pain influences the extent to which autonomic arousal mediates the effects of stimulus intensity on pain

Authors: D. MISCHKOWSKI[1], E. PALACIOS-BARRIOS[1], L. A. BANKER[1], T. C. DILDINE[1], *L. Y. ATLAS[2,1];
[1]Natl. Ctr. for Complementary and Integrative Hlth., NIH, Bethesda, MD; [2]NIH (NCCIH, NIDA), Bethesda, MD

Abstract: Skin conductance represents a cardinal measure of autonomic arousal and, as such, consistently correlates with self-reports of physical pain in response to noxious stimulation (e.g., Geuter, Gamer, Onat, & Büchel, 2014; Loggia, Juneau, & Bushnell, 2011). However, it remains unclear whether increased autonomic arousal during pain reflects objective stimulus proprieties (i.e., stimulus intensity) or stimulus-independent psychological factors, such as negative affect in anticipation of noxious stimulation. If pain-related affective processes beyond objective stimulus intensity influence autonomic arousal, (1) a stronger disposition for fear in response to pain should influence skin conductance responses (SCR) following noxious input, and (2) SCR should predict trial-by-trial pain reports, controlling for noxious input. In addition, these relationships may be influenced by individual differences known to modulate pain. We tested whether SCR mediates the effects of stimulus intensity (i.e. temperature) on pain and whether dispositional fear of pain (McNeil, Rainwater, & Aljazireh, 1986) moderated the effect of stimulus intensity on SCR. Participants (N=59) received 24 trials of low, medium, and high painful thermal stimulation and provided pain ratings on every trial following heat offset. We measured SCRs evoked during noxious stimulation. Using multilevel mediated moderation to account for hierarchical data structure, we found that a) SCR increased as a function of temperature, consistent with previous work (z=6.71, p<.001), b) dispositional fear of pain increased the association between temperature and SCR (z=2.06, p<.05), c) SCR predicted pain ratings independent of temperature (z=4.57, p<.001), and d) SCR mediated the effect of temperature on reported pain (z=3.90, p<.001). These findings suggest that a) stimulus-independent fluctuations in affective processes influence pain; b) these processes can be measured with SCR; and c) stimulus intensity effects on SCR are influenced by fear of pain. More research is needed to isolate the effects of specific underlying psychological factors (e.g. attention, emotion, and expectation) that give rise to fluctuations in SCRs during pain and to investigate the utility of autonomic measures as biomarkers for pain.

Disclosures: D. Mischkowski: None. **E. Palacios-Barrios:** None. **L.A. Banker:** None. **T.C. Dildine:** None. **L.Y. Atlas:** None.

Poster

525. Pain Models: Physiology

Location: Halls B-H

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Program#/Poster#: 525.04/RR13

Topic: D.02. Somatosensation: Pain

Support: NHMRC

Title: Biological activity of alanine, lysine and aspartic acid-substituted analogues of α-conotoxin Vc1.1 on high voltage-activated calcium channels in mice DRG neurons

Authors: *M. SADEGHI[1], B. CALLAGHAN[2], R. J. CLARK[3], D. J. ADAMS[1];
[1]Illawarra Hlth. and Med. Res. Institute,, University Of Wollongong, Australia; [2]Anat. and Neurosci., The Univ. of Melbourne, Melbourne VIC 3010, Australia; [3]Sch. of Biomed. Sci., The Univ. of Queensland, Brisbane QLD 4072, Australia

Abstract: α-Conotoxins are peptides obtained from cone snail venoms and are characterized by their affinity for nicotinic acetylcholine receptors (nAChR). They are distinguished by two disulfide bonds that have Cys^{I}-Cys^{III} and Cys^{II}-Cys^{IV} connectivity with a varying number of residues within these two loops. Several α-conotoxins with distinct selectivity for nAChR subtypes have been identified as potent analgesics in animal models of neuropathic and chronic pain. Analgesic α-conotoxins, Vc1.1 and Rg1A, are selective α9α10 nAChR antagonists, however, some Vc1.1 analogues which potently antagonized α9α10 nAChR, had no analgesic effect in rat models of neuropathic pain (Nevin et al., 2007, Mol pharmacol 72, 1406-1410). We showed that α-conotoxin Vc1.1 and Rg1A inhibited N-type Ca^{2+} channel currents in rat dissociated dorsal root ganglion (DRG) neurons via activation of G protein-coupled $GABA_B$ receptors ($GABA_BR$) (Callaghan et al., 2008, J Neurosci 28, 10943-10951; Callaghan et al., 2010, Channels 4, 51-54). Scanning mutagenesis revealed the key residues crucial for biological activity of α-conotoxins Vc1.1 and RgIA on α9α10 nAChRs (Halai et al., 2009, J Biol Chem 284, 20275-20284). Therefore, in the present study we investigated the structure–activity relationships between α-conotoxin Vc1.1 and $GABA_BR$. The residues except the conserved cysteines contained in the amino acid sequence of Vc1.1, were subsequently replaced by alanine (A), lysine (K) and aspartic acid (D). We examined the activity of Vc1.1 analogues on high voltage-activated (HVA) Ca^{2+} channel currents in mice DRG neurons using the whole-cell patch clamp recording technique. Alanine substituted analogues of Vc1.1 exhibited reduced potency and efficacy at $GABA_BR$-mediated inhibition of HVA Ca^{2+} channels except where alanine substituted amino acids in second loop at positions Asp^{11}, Glu^{14} and Ile^{15} of Vc1.1. These analogues retain activity at $GABA_BR$ and potently inhibited Ca^{2+} current but lost their activity at α9α10 nAChR. In contrast, [N9A]Vc1.1 was inactive at $GABA_BR$ but retained activity at α9α10 nAChR whereas [N9K]Vc1.1 was active at $GABA_BR$. These results suggest that the small

hydrophobic residues at position 9 increase the potency at nAChR whereas the positive charged residues at this position increase the selectivity for $GABA_BR$ over nAChR. Therefore, the residues in the second loop of Vc1.1 play an important role in the selectivity for $GABA_BR$ activation whereas those in the first loop are important for binding to the receptors. These findings enhance our understanding of the key residues important for binding of Vc1.1 at $GABA_BR$ and the selectivity for $GABA_BR$ over α9α10 nAChRs.

Disclosures: **M. Sadeghi:** None. **B. Callaghan:** None. **R.J. Clark:** None. **D.J. Adams:** None.

Poster

525. Pain Models: Physiology

Location: Halls B-H

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Program#/Poster#: 525.05/RR14

Topic: D.02. Somatosensation: Pain

Support: NMRC R185-000-247-511

Title: The posterior hypothalamus modulates pain behaviors in the formalin model of acute inflammatory pain

Authors: ***Z. WANG**, M. ARIFFIN, T. SOONG, S. KHANNA;
Natl. Univ. of Singapore, Singapore, Singapore

Abstract: In the present study we have explored the role of posterior hypothalamic (PH) regions in nociception. The region is interconnected with limbic and brainstem areas that are associated with affect-motivation, at least in part. Indeed, the lateral supramammillary nucleus (lSuM) and the ventral mammillary bodies (MB) of the PH are associated with the septo-hippocampal network which is implicated in facilitation of nociception. Inactivation of the lateral SuM (lSuM) blocked hippocampal nociceptive responses induced on hind paw injection of formalin, an algogen, in anaesthetized animal. We, thus, hypothesized that the PH is involved in nociception. So as to test the hypothesis, the effects of attenuation of neural activity along the dorso-ventral axis of the PH was tested on acute behaviors evoked on hind limb injection of formalin. The neural activity was attenuated by microinjection of muscimol (2ug/ul, 0.1ul), a GABA mimetic, or NBQX (10ug/ul, 0.1ul), a receptor antagonist at the glutamatergic AMPA receptors, into the lSuM or the MB.
Microinjection of the drugs evoked a site-dependent effect on nociception. A weak effect was observed with microinjection into the lSuM, whereas microinjection into the MB evoked a strong effect. In this regard, microinjections of muscimol or NBQX into lSuM attenuated flinching behavior in the second phase (11-60mins after hind paw formalin injection) of the formalin test,

without affecting licking responses. Neither did muscimol-microinjection affect the power of the hippocampal theta observed during the formalin test, theta being 3-12 Hz rhythmic sinusoidal extracellular waveform that reflects neural processing of information. However, the dose administered was sufficient to attenuate reticular stimulation-evoked hippocampal neural responses in anesthetized rat. The reticular stimulation-evoked hippocampal responses are known to be mediated via the lSuM.
On the other hand, microinjections of both muscimol and NBQX into MB resulted in suppression of flinching and licking behaviors, the effect of NBQX being quite marked. The behavioral effect of muscimol-microinjection into MB was accompanied by reduction in the amplitude of theta wave activity recorded from the hippocampus.
Collectively, the foregoing data point towards functional differences along the dorso-ventral axis of the PH such that the ventral MB play a much greater role in acute nociception in the formalin test. Indeed, the findings suggest a role of glutamatergic transmission in MB in mediation of nociception.

Disclosures: **Z. Wang:** None. **M. Ariffin:** None. **T. Soong:** None. **S. Khanna:** None.

Poster

525. Pain Models: Physiology

Location: Halls B-H

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Program#/Poster#: 525.06/RR15

Topic: D.02. Somatosensation: Pain

Support: Dept. Defense W81XWH-16-1-0071

VA-ORD (RR&D) 1IO1RX002101

Title: Characterization and validation of photosensitivity as a biomarker of post-traumatic headache in mice.

Authors: ***A.-S. WATTIEZ**, L. P. SOWERS, B. J. REA, T. C. YIN, A. KUBURAS, A. F. RUSSO;
Mol. Physiol. & Biophysics, Univ. of Iowa, Iowa City, IA

Abstract: The prevalence of post-traumatic headaches (PTH) in military personnel who have been diagnosed with traumatic brain injury (TBI) reaches 59%, leading to a severe disruption of the daily activities, ability to work and quality of life of thousands of Veterans. Although extremely prevalent and debilitating, the pathophysiological mechanisms underlying PTH remain unknown, and the efficacy of existing treatments is poor. PTH presents symptom similarities

with chronic migraine, including a high prevalence of photosensitivity in patients both during and in between headaches, which makes it a practical biomarker of both conditions. The goal of the present study is to characterize and validate photosensitivity as an objective biomarker of PTH in a mouse model, which would enable us to test future treatment strategies pre-clinically. In order to model TBI, the heads of the mice are exposed to an over-pressure chamber while under anesthesia. In order to mimic the multiple head injuries that some soldiers can receive, we compared the effect of sham-injured mice with mice receiving 1 injury, or 3 injuries over 90 minutes or 1 week. In those animals, photophobia was measured within two assays. In the light aversion assay, the animals can freely choose between a light and a dark zone. This test was run in parallel with two tests in order to control for activity and anxiety: the open-field assay and the elevated plus-maze assay. In the eyelid closure assay, the squinting of mice in the different groups was scored and compared in the dark and in response to exposure to bright light.
Our preliminary data show that expositions to an over-pressured chamber are able to produce photophobia in mice, as compared with their sham littermates. Those animals do not show anxiety or decrease of activity at 5 weeks after the injury. Present results need to be repeated and extended to later time-points. If confirmed, those findings will support the validity of photosensitivity as an objective biomarker of post-traumatic headache, and will allow us to study the mechanisms behind the injury, as well as screen for future PTH treatments.

Disclosures: **A. Wattiez:** None. **L.P. Sowers:** None. **B.J. Rea:** None. **T.C. Yin:** None. **A. Kuburas:** None. **A.F. Russo:** None.

Poster

525. Pain Models: Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 525.07/RR16

Topic: D.02. Somatosensation: Pain

Support: NIH SC2 GM095428-0181

Title: cAMP and cGMP attenuate nociceptive sensitization *In vivo* in a novel model organism, M. sexta

Authors: *F. E. ARREOLA[1], C. MOFFATT[2], M. FUSE[2];
[1]Biol., [2]San Francisco State Univ., San Francisco, CA

Abstract: Nociception describes the process of encoding the response to potential or damaging stimuli by the central nervous system, which is noted as pain in higher organisms. Nociceptive sensitization is a form a non-associative learning that can persist for days or months in animals,

where the noxious stimulus elicits a stronger response or where an otherwise innocuous stimulus now generates a response. Genetic analysis of different species suggests a conservation of nociceptors and signaling molecules such as soluble guanylyl cyclase, and protein kinases typically involved in sensitization. However how these cellular factors mediate and modify nociceptive cells in different animals remains unclear. Research suggests that cAMP and cGMP, both second messengers, have roles in nociceptive sensitization, but results differ between and within organisms (e.g. central vs peripheral effects). cGMP is often the product of soluble guanylyl cyclase, typically activated by NO. Using a novel organism to study nociception - *Manduca sexta,* commonly known as the Tobacco hornworm - we tested the actions of cGMP and cAMP in modulating nociceptive sensitivity. We used the modified Simplified Up Down (SUDO) method to assess the threshold of a defensive strike behavior that occurs after a noxious insult. We then assessed nociceptive sensitivity after injection of the membrane permeable cGMP and cAMP analogs, 8-Bromo-cGMP and 8-Bromo-cAMP, respectively, as well as the soluble guanylyl cyclase inhibitor Methylene Blue. Injections of 8-Bromo-cGMP and 8-Bromo-cAMP each had an analgesic effect on animals that had a noxious stimulus applied, in that sensitivity was reduced in the presence of the exogenous compound. Animals treated with Methylene Blue demonstrated increased sensitivity with or without receiving a noxious stimulus. Our data suggests that cGMP and cAMP may mediate analgesic effects in *M. sexta*. *M. sexta* appears to be a suitable new model to study nociceptive sensitization that may offer unique advantages over other model organisms.

Disclosures: F.E. Arreola: None. **C. Moffatt:** None. **M. Fuse:** None.

Poster

525. Pain Models: Physiology

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Program#/Poster#: 525.08/RR17

Topic: D.02. Somatosensation: Pain

Support: NRF-2015M3C8A5060108

Title: Analgesic effect of Oxytocin was attributed to AVP1a receptor-mediated hyperpolarization of dorsal root ganglion cells

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Abstract: Recent studies have provided several lines of evidence that peripheral administrations of OT-induced analgesia in human and rodents. However, the exact underlying mechanism of the analgesia still remains elusive. In the present study, we investigated 1) to identify which receptor mediated analgesic effect of OT, 2) to see whether OT affected capsaicin-induced intracellular calcium transients, the membrane excitability and capsaicin-induced current of TRPV1 channel. If so, we examined 3) which receptor mediated these changes. Lastly we interrogated 4) to verify the expression patterns of mRNA of these receptors.
For these aims, we examined whether the intraperitoneal (IP) injection of specific antagonist for OT and AVP1a receptors reversed the OT-induced analgesia during the pain behavioral tests such as Hargreaves' and capsaicin injection tests in rats. We explored the effect of OT and its antagonists on capsaicin-induced intracellular calcium transient using fluorescent microscopy in the dissociated dorsal root ganglion (DRG) cells. In addition, we investigated the effect of OT and its antagonists on the membrane excitability and the capsaicin-induced current of TRPV1 channel using patch clamp in the dissociated DRG cells. Further, we determined mRNA expression patterns of TRPV1 and the oxytocin related receptors in the individual cells by single cell RT-PCR.
The behavioral results showed that the antagonist for AVP1a receptor reversed the OT-induced analgesia. The fluorescent imaging experiments demonstrated that OT partially inhibited capsaicin-induced intracellular calcium transient through AVP1a receptor in the DRG cells. The electrophysiological study also demonstrated that OT hyperpolarized the membrane potential and reduced the current of TRPV1 channel through AVP1a receptor in the DRG cells. Furthermore, single cell RT-PCR discovered co-expression of mRNA of TRPV1 and AVP1a receptor in the DRG cells. Also, the number of cell expressing mRNA of AVP1 receptor was much more than that of OT, AVP1b, and AVP2 receptors.
Taken together, our findings possibly suggested that OT-induced membrane hyperpolarization of the DRG cells contributed to the analgesic effects of peripheral administration of OT through AVP1a receptor.
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Disclosures: **T. Han:** None. **H. Na:** None. **H. Kim:** None. **S. Back:** None. **H. Kim:** None. **S. Oh:** None.

Poster

525. Pain Models: Physiology

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 525.09/RR18

Topic: D.02. Somatosensation: Pain

Title: Is it possible for pro-inflammatory cytokine in intervertebral disc to induce discogenic low back pain ?

Authors: *E. PARK, S. MOON, H. SUH, H. HAN;
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Abstract: Intervertebral disc (IVD) can be a major source of low back pain (LBP). Some studies reported that in degenerated IVDs the upregulation of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β) or tumor necrosis factor-alpha (TNF-α) can promote nerve fiber to grow into the deep layer of annulus fibrosus and give rise to painful IVD. However, it is not clear whether pro-inflammatory cytokines in IVDs can participate in nociceptive processing. The purpose of this study was to characterize the responses of mechanosensitive afferent (MSA) in IVD after the intradiscal injection of IL-1β or TNF-α. We previously identified that the paravertebral sympathetic trunks (PST) of male SD rats innervated lumbar IVD through DiI labeling (2μl; a lipophilic and fluorescent dye). Using male SD rats (300-350g; Korea), PST was teased into small strands and placed on a unipolar platinum electrode in warm mineral oil pool, and *in vivo* single nerve recording of the IVD afferent was done before and 30 min after intradiscal injection of IL-1β (1 or 10ng/ml) or TNF-α (1ng/ml). Drugs (3μl) were delivered through 25G needle and tubing connected to a Hamilton syringe, and MSA activities (maximum spikes per second) stimulated by von Frey filament (0.4 to 10g) were counted. Compared to saline-injection group, MSA activities in IL-1β or TNF-α-injected group significantly increased 30 min post-drugs injection time. The present study implicate that the nociceptive information from IVDs can be transmitted to spinal cord through paravertebral sympathetic pathways, and the upregulation of pro-inflammatory cytokines in degenerated IVD can induce discogenic pain.
Keywords: intervertebral discs (IVDs), pro-inflammatory cytokines, mechanosensitive afferent, *in vivo* single nerve recording

Disclosures: **E. Park:** None. **S. Moon:** None. **H. Suh:** None. **H. Han:** None.

Poster

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Program#/Poster#: 525.10/RR19

Topic: D.02. Somatosensation: Pain

Support: Telethon n. GGP11179

FP7 PAINCAGE project n. 603191

Title: Phenotypic characterization of a mouse model of Hereditary Sensory and Autonomic Neuropathy type V: new central aspects of feeling pain

Authors: G. TESTA[1], L. PANCRAZI[1], M. CECI[1,2], C. MORELLI[3], M. FABBRI[1], M. COSTA[4], P. HEPPENSTALL[3], A. CATTANEO[1,2], *S. CAPSONI[1];
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Abstract: The Hereditary Sensory and Autonomic Neuropathy type V (HSAN V) is linked to defects in Nerve Growth Factor (NGF) signaling. HSAN V is clinically characterized by absence of pain sensation leading to painless fractures, bone necrosis, osteochondritis, and neuropathic joint destructions (Capsoni, 2014). The genetic mutation responsible for the disease is the R100W point mutation (661C>T) in the NGF gene (Einarsdottir et al., 2004). HNGF R100W is a preferential TrkA biased agonist (Covaceuszach et al 2010; Capsoni et al 2011). We confirmed the finding by Larsson et al. (2009) that the R100W mutation inhibits processing of proNGF to mature NGF, with an accumulation of proNGF in cultured cells. To understand the mechanisms at the basis of this disease, we have generated two knock-in mouse lines in which the murine NGF coding sequence is replaced by the coding sequence of human NGF, either in its wild type or R100W forms. Haploinsufficiency of mature NGF could be the cause of death of homozygous HSAN V mice occurring during the first month of life. To continue the characterization of the effects of HSAN V mutation during adulthood, we performed a combination of molecular, neuroanatomical and behavioral analyses in heterozygous mice. Due to the known effects of NGF on learning and memory, we analyzed heterozygous mice for the presence of deficits in object recognition and spatial memory tests and found no impairment in both tasks. At two months, heterozygopus mice show no deficits in thermal perception, anxiety and “cage behavior”, which, however, are impaired in 6 month-old heterozigous HSAN V mice. At all ages, the R100W mutation causes less sensitivity to pain induced by capsaicin injection, in dosage and sex-dependent manner. The insensitivity to pain might interfere with learning and memory of painful events or with their interpretation as painful. Thus, in the fear conditioning test, we increased the potency of the aversive stimulus with respect to wild type mice and found

that, despite the perception of the aversive stimulus, HSAN V mice do not show a fear response. Interestingly, the late onset of the phenotype and the inability to mount aversive responses to pain accurately model the clinical phenotype of heterozygous patients (Minde et al., 2009). In conclusion we were able to reproduce in mice the main clinical HSAN V phenotype. Further study of this model will allow to expand our knowledge on the pro-nociceptive actions of NGF, from peripheral sensory system to central mechanisms of pain perception and is providing new insights into the central consequences of growing without feeling pain.

Disclosures: **G. Testa:** None. **L. Pancrazi:** None. **M. Ceci:** None. **C. Morelli:** None. **M. Fabbri:** None. **M. Costa:** None. **P. Heppenstall:** None. **A. Cattaneo:** None. **S. Capsoni:** None.

Poster

525. Pain Models: Physiology

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Program#/Poster#: 525.11/RR20

Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS 075599

Title: Induction of photophobic behavior in mice by peripheral CGRP: Implications for migraine therapy

Authors: ***B. N. MASON**[1], A. KUBURAS[1], L. GARCIA-MARTINEZ[2], J. A. LATHAM[2], A. F. RUSSO[1];
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Abstract: The neuropeptide calcitonin gene-related peptide (CGRP) has been firmly established as a key player in the pathophysiology of migraine. However, there is controversy brought to the forefront by the recently reported efficacy of CGRP-blocking antibodies in clinical trials as to the relative contributions of CGRP in the periphery versus the central nervous system. We have previously documented that light aversion in mice can be used as a surrogate for photophobia in response to central ICV administration of CGRP. In this study, we confirm these central mechanisms and report that two strains of wildtype mice (C57Bl/6J, CD1) show light aversive behavior in bright light (27,000 lux) following peripheral I.P. administration of CGRP. As with central administration, the phenotype was not solely due to increased anxiety based on a light-independent anxiety assay. In addition, motility was decreased in the dark zone, but not in the light. Importantly, both sumatriptan, a 5-$HT_{1B/D}$ migraine drug, and a CGRP monoclonal antibody attenuated CGRP-induced light aversion. To address the site(s) of CGRP action, we used a genetic strategy with a transgenic strain of CGRP-sensitized mice that have elevated

hRAMP1 CGRP receptor subunit in nervous tissue (nestin/hRAMP1). These mice failed to exhibit light aversion with low levels of light (55 lux) after peripheral injection of CGRP; this in contrast to the ability of a central injection of CGRP to elicit light aversion in these mice. These results suggest that CGRP can act both in the periphery and the brain to induce light aversive behavior in mice but through different mechanisms.

Disclosures: B.N. Mason: None. **A. Kuburas:** None. **L. Garcia-martinez:** None. **J.A. Latham:** None. **A.F. Russo:** None.

Poster

525. Pain Models: Physiology

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant NS075599

Title: Peripheral injection of PACAP38 induces photophobia in mice

Authors: A. KUBURAS[1], B. N. MASON[1], L. P. SOWERS[1], M.-C. MOLDOVAN LOOMIS[2], L. F. GARCIA-MARTINEZ[2], J. A. LATHAM[2], *A. F. RUSSO[1];
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Abstract: Migraine is a disabling neurological disorder and a significant public health problem. The pathophysiology of migraine is not well understood, but studies have shown that calcitonin gene-related peptide (CGRP) plays a strong role. In recent years there has been a significant interest for role of pituitary adenylate cyclase-activating polypeptide-38 (PACAP38) in migraine. The objective of this study is to determine if administration of PACAP38 will induce photophobia, a migraine-like symptom, in wild-type CD1 mice. As a surrogate to photophobia, we measured light aversion behavior. Our results show that intraperitoneal injection of PACAP38 induced a significant light-aversive response in CD1 mice compared to mice treated with vehicle. In addition, PACAP38 also caused an increase in resting time in the dark reflecting a possible aggravation of pain by movement as experienced during migraine. Future work will attempt to understand more about PACAP38 and possible connections with CGRP in migraine and to explore PACAP as a potential novel target in migraine therapy.

Disclosures: A. Kuburas: None. **B.N. Mason:** None. **L.P. Sowers:** None. **M. Moldovan Loomis:** A. Employment/Salary (full or part-time): Alder Biopharmaceuticals. **L.F. Garcia-**

Martinez: A. Employment/Salary (full or part-time): Alder Biopharmaceuticals. **J.A. Latham:** A. Employment/Salary (full or part-time): Alder Biopharmaceuticals. **A.F. Russo:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Alder Biopharmaceuticals. F. Consulting Fees (e.g., advisory boards); Alder Biopharmaceuticals.

Poster

525. Pain Models: Physiology

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Topic: D.02. Somatosensation: Pain

Support: NIH NINDS NS035115

NIDCR DE022746

Title: A flexible model for characterizing sub acute and chronic back pain trajectories and etiology

Authors: *B. PETRE, A. APKARIAN;
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Abstract: Distinct brain physiology in subacute back pain (SBP; pain 4-16 weeks) is associated with different pain outcomes one year later, and suggests high motivational load underlies pain persistence. Our aim is to develop a formal definition of these outcomes by modeling pain trajectories. We pursue this using a previously published group of 59 SBP patients, encountered up to 8 times over the course of a year, who received T1 and diffusion imaging scans at study entry. We apply our model to sparse subsets of this data to demonstrate its flexibility. Parsimonious subdivisions of pain trajectories are identified by fitting a Gaussian mixture model to visual analog scale pain ratings. Four roughly equal subdivisions are obtained, matching previously identified subacute and chronic back pain trajectories. Normalizing by percent change in pain suggests a more parsimonious model preserving one of the preliminary recovery-like subgroups (N=14) and aggregating the rest (42) who show persistent pain (3 outliers remain). Using hierarchical nonlinear Bayesian regression we construct a probabilistic latent model of these trajectories which we use to parameterize Naive Bayesian classifiers of novel pain trajectories measured at arbitrary timepoints. We evaluate the classifier based on within-subject subsamples of pain reports and obtain high classification accuracies (AUC=0.8-0.9) using either randomly or systematically selected singular follow-up pain reports, although subject level

confidence increases with additional data.
Two exemplary biomarkers of pain persistence previously identified in this dataset, hippocampal volume and fractional anisotropy (FA), were investigated for correspondence to specific pain trajectories. As expected, biomarkers differ significantly between pain trajectory clusters (FA: t-test, $p = 0.015$, volume: t-test, $p = 0.021$). More notably, pain report data at a randomly selected follow up can achieve classifications with similar differences in brain etiology (FA $p = 0.013$, volume not significant) while explaining 74% of the variance in posterior probabilities obtained with the full pain trajectory dataset.
These findings show persistence and recovery from SBP can be formally assessed using a simple classifier on pain rating data collected at arbitrary timepoints, which can be flexibly applied to novel experimental or clinical settings.

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Poster

525. Pain Models: Physiology

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 525.14/SS1

Topic: D.02. Somatosensation: Pain

Title: Cortical mechanisms of pain processing in non-human primates

Authors: *B. J. HANSEN, J. D. VARDIGAN, S. J. TYE, S. V. FOX, J. M. USLANER;
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Abstract: Traditional ways of measuring analgesia clinically rely on subjective reports of pain, which cannot be measured preclinically in animals. Over the past decade, the importance of developing objective, quantifiable experimental pain models has been acknowledged in the clinical setting; however, there has been little preclinical work towards this effort. For example, clinical research has established a model in which electrical activity in the brain is measured during application of painful heat stimulation to the forearm. To this end, we have developed a novel and highly translatable experimental paradigm to record electroencephalography (EEG) activity from multiple cortical locations and measure event-related potentials (ERPs) in awake-behaving primates during heat stimulation. Briefly, four female rhesus macaques (*Macaca mulatta*) were implanted with a PhysioTel D70 (Data Sciences International) capable of wirelessly transmitting EEG activity from three distinct cortical regions corresponding to F3, C3 and P3. Heat stimulation was controlled using a contact heat-evoked potential stimulator (CHEPs; Medoc Ltd.) with a round thermode that contacts a cutaneous area of 572.5 mm^2. This device reliably delivered temperatures with a heating rate of 70°C/s. On a given trial, heat

stimulation at either 44 or 50°C was applied to the animal's right volar forearm for 0.5 s followed by a 30 s ITI. Each temperature was randomly presented 50 times in a given session. The data were pre-processed using custom software coded and complied in Matlab according to the following steps: (1) artifact rejection (e.g. high amplitude trials); (2) baseline subtraction of the average activity 200 ms prior to trigger onset; (3) Savitzy-Golay filter with polynomial order of three. For each channel and temperature we measured the negative (N_2; 200-400 ms) and positive (P_2; 300-500 ms) potentials from baseline to peak and calculated the peak-to-peak (N_2-P_2) amplitude. Temperature specific changes were observed in N_2-P_2 amplitude (44: mean +/- SEM, 11.61 +/- 1.42μV; 50: mean +/- SEM, 17.17 +/- 2.58μV). Interestingly, this effect was strongest on P3, the EEG channel located over the Inferior Parietal Lobe, an area involved in the interpretation of sensory information. Now there is an unprecedented opportunity to study the cortical mechanisms of pain processing in non-human primates, which is directly translatable to the clinic. This will allow us to assess the role of analgesic compounds and their influence on cortical activity in a large animal species in a preclinical setting.

Disclosures: **B.J. Hansen:** A. Employment/Salary (full or part-time): Merck & Co. **J.D. Vardigan:** A. Employment/Salary (full or part-time): Merck & Co. **S.J. Tye:** A. Employment/Salary (full or part-time): Merck & Co. **S.V. Fox:** A. Employment/Salary (full or part-time): Merck & Co. **J.M. Uslaner:** A. Employment/Salary (full or part-time): Merck & Co..

Poster

525. Pain Models: Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 525.15/SS2

Topic: D.02. Somatosensation: Pain

Support: VA Merit Review grant

Title: Cerebral cholinergic mechanisms in pain: CBF lesions vs systemic scopolamine

Authors: ***R. G. WILEY**[1], R. YEZIERSKI[2], C. J. VIERCK, Jr[3];
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Abstract: Cholinergic inputs to the cerebral cortex and limbic system, originating primarily from the cholinergic basal forebrain (CBF), play an important role in cortical sensory processing, largely through modulation of inhibitory interneurons. Cholinergic agonists given spinally, intracerebroventricularly (ICV) or systemically depress reflex nocifensive responses, but systemic cholinergic **antagonists** also depress some affective responses to pain and impair

attention to aversive stimuli and stress reactions. In the present study, we determined the effects of selective cerebral cholinergic denervation, using ICV microinjection of 4 ug of 192-saporin in 10 µl (Advanced Targeting Systems, San Diego, CA) on operant thermal escape responses to aversive thermal stimuli (10° C, 44.5° C) and hyperalgesic effect of sound stress (ten X 30 sec bursts of 100 dB white noise over a 15 min period, 20 mins prior to thermal escape testing) in normal and CBF-lesioned rats compared to effects of systemic cholinergic antagonism (0.1 mg/kg, i.p., scopolamine, 20 minutes prior to thermal escape testing) in intact, normal rats. All rats were on the thermal escape task prior to either scopolamine, or sound stress testing and prior to ICV 192-saporin. At the conclusion of behavioral testing, choline acetyltransferase immunohistochemistry confirmed that 192-sap produced 62-81% loss of CBF cholinergic neurons. CBF-lesioned rats showed **decreased** thermal escape responses to both temperatures (10°C and 44.5°C) for >19 weeks. There also was no increase in escape responding (hyperalgesia) after sound stress as seen in normal rats. Scopolamine in normal rats produced decreased thermal escape responses to cold (2° C, 6°C and 10° C) and to heat (44.5° C). These results suggest that systemic scopolamine mimics the effects of CBF destruction on pain and together the overall results are interpreted to indicate an important role for the CBF in cerebral pain processing. These findings may be relevant to clinical pain care in patients with cerebral cholinergic dysfunction, such as Alzheimer's disease.

Disclosures: R.G. Wiley: None. **R. Yezierski:** None. **C.J. Vierck:** None.

Poster

525. Pain Models: Physiology

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Program#/Poster#: 525.16/SS3

Topic: D.02. Somatosensation: Pain

Support: DOD W81XWH-16-1-0071

VA-ORD1IO1RX002101

Title: Detection of CGRP-induced pain by a facial response assay in a mouse migraine model

Authors: *B. J. REA[1], J. L. BITTLE[1], L. TERAN[1], P. POOLMAN[2], W. A. JOHNSON[1], L. P. SOWERS[1], R. H. KARDON[2], A. F. RUSSO[1];
[1]Mol. Physiol. & Biophysics, [2]Oph and Visual Sci., Univ. Iowa, Iowa City, IA

Abstract: Migraine is a complex neurological disorder that afflicts over 6% of men and 18% of men in the United States. Having a myriad of symptoms, migraine is denoted by debilitating,

unilateral pain, lasting up to 72 hours, and at least one of two symptoms: nausea and/or vomiting, or photophobia and phonophobia. Photophobia is a condition where low to normal levels of light cause discomfort and light aversion in the perceiver. This photosensitivity is a subjective experience for each migraineur and is a common trigger.
Calcitonin gene-related peptide (CGRP) is a neuropeptide that is elevated during migraine. Clinical evidence suggests that CGRP plays a key role in migraine etiology. In particular, intravenous injection of CGRP has been shown to induce migraine-like headache in migraineurs but only fullness-of-head in non-migraineurs. Currently we have an established mouse model for CGRP-induced photophobic behavior. However, we have yet to quantify pain expression post CGRP administration. We hypothesized that our mice would exhibit increased expression of pain after CGRP administration and that this expression may be increased in the presence of light. Mice were acclimated to a customized restraint and recorded using multiple cameras during dark and light conditions. Mice were then given an intraperitoneal injection of CGRP (0.1-0.5 mg/kg) or saline and underwent the same conditions. Using the Mouse Grimace Scale and point-to-point measurement software, mice were independently scored by blinded observers for pain expression.
CGRP caused a significant increase in pain expression compared to saline control in both dark and light conditions. A difference between dark and light was not observed. Thus, CGRP causes a facial pain response in mice that is consistent with the causal role of CGRP in migraine.

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Poster

525. Pain Models: Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 525.17/SS4

Topic: D.02. Somatosensation: Pain

Title: Reduced PFC activity may underlie reduced attentional performance from incisional pain

Authors: *D. RIRIE, D. BOADA, T. MARTIN;
Wake Forest Univ., Winston Salem, NC

Abstract: Introduction. Cognitive capacity may be reduced from exposure to inflammation, surgery, anesthesia, and pain (1-2). Attentional performance can be measured serially in the rat using the 5 Choice Serial Reaction Titration Variant (5CTV) and surgical incision impairs attentional performance (3,4). In this study, we hypothesized that a reduction in local field potentials (LFP) in the medial prefrontal cortex (mPFC) occurs in response to incision and

correlates with reduced attentional performance.
Methods. After Institutional Animal Care and Use Committee Approval, 8 Male Fisher 344 Rats were trained in the 5CTV (4). After a stable titration of cue duration (a measure of attention is the median cue duration (MCD)) to < 1 second, animals underwent anesthesia for placement of electrodes into the mPFC. After return of the MCD to <1 s, LFP and MCD were measured in all animals before and on postop day (POD) 1 and 3 after paw incision (5). Change in LFP power spectral density (PSD) was measured. Data were analyzed using repeated measures ANOVA to test significance with corrections for multiple comparisons. Results. The MCD is increased on POD 1 and 3 compared to baseline prior to incision consistent with worse performance ($p<0.05$). Average PSD from 0-50 Hz in the mPFC was reduced on POD1 and POD3 ($p<0.05$) (Figure 1: Power Spectral Density Change after Incision). The reduced LFP power in the mPFC parallels the decrease in attentional performance measured by the increase in MCD that occurs from surgery. Discussion. The reduction in mPFC synaptic activity as measured by a local decrease in LFP power may underlie the inability to maintain attention and to perform maximally. Furthermore, the effects of anesthesia and pain on mPFC cortical activity extend beyond the day of anesthesia and surgery and may reduce attentional performance for days after a small procedure. Pain may alter attentional performance by increasing attentional load and disrupting information processing and altering input (6). Although the MCI related attentional dysfunction resolves, the ramifications for persisting beyond the immediate recovery from anesthesia are large. MCI may impair recovery time from the standpoint of return to optimal functioning in daily activities, particularly activities with higher risks requiring maximal attention and optimum performance for the greatest safety for the patient and those affected by their performance.
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1. Lancet. 1998; Mar 21;351(9106):857-61.
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3. Journal of Neuroscience Methods 2015; 241:37-43.
4. ASA 2015; A4011.
5. Anesthesiology. 2003; Aug;99(2):443-8

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Poster

525. Pain Models: Physiology

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Topic: D.02. Somatosensation: Pain

Support: CIHR RES0023156

University Hospital Foundation RES0029278

NSERC RES0007116

Title: Nile rats as novel model of protracted type-2 diabetes-induced peripheral sensory neuropathy

Authors: *J. SINGH[1], A. RUANGKITTISAKUL[1], P. SHELEMEY[2], T. JOY[3], J. SCHNEIDER[1], Y. SAUVE[1], K. BALLANYI[1], C. WEBBER[3];
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Abstract: A devastating complication of type-2 diabetic (T2D) is the development of peripheral sensory neuropathy (PSN) typified by mechanical allodynia, hyperalgesia, spontaneous burning pain, thermal hypersensitivity and numbness in lower extremities. Current animal models have provided insights on the contribution of nociceptor (A-delta and C-fibers) sensitization to T2D neuropathic pain; however, genetic complexity, alteration of the biological system, and rapid T2D progression makes these animals models difficult to select for and mimic human T2D pathogenesis at preclinical stages. Recently, Nile rats fed standard laboratory rodent chow (Prolab 2000) were shown to develop hyperinsulinemia at 2 months of age followed by hyperglycemia at 6 months with ultimate decompensation (islet beta cell loss and inability for systemic glucose retention) by 18 months. We relied on these animals to study whether they also demonstrate evidence of neuropathic pain by comparing them to age-matched controls obtained by feeding an independent group of Nile rats a higher fibre low calorie diet (Mazuri). This group of animals (controls) did not develop hyperglycemia up to 18 months of age. Here, we show using a saphenous skin-nerve preparation (Zimmermann et al [2009] Nature Prot. 4:174-196) that 18 month T2D-NGR A-delta and C fibers have decreased electrical and mechanical (Von-Frey test) excitation thresholds. Similar to human patients, Nile rats have a reduced number of intra-epidermal pain-sensing nerve fibers (immunolabeled for pan-axonal marker protein gene product 9.5) in the footpad, when compared to controls. Furthermore, anatomical changes take place in both A-delta and C fiber as documented by immunolabeling at their cell bodies in the dorsal root ganglion neurons (with anti-calcitonin gene-related peptide and anti-Isolectin B4 antibodies, respectively). Our findings indicate that Nile rats fed standard rodent chow develop pathological features of T2D-induced peripheral sensory neuropathy that have analogies with the human pathogenesis. This novel model opens long waited and needed opportunities to undertake preclinical studies for diabetic neuropathy.

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Poster

525. Pain Models: Physiology

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: P20GM103643

Title: Investigating primary cilia in nociceptive dorsal root ganglion neurons and their potential links to acute and chronic pain.

Authors: *K. L. LINDROS, E. J. BILSKY, K. L. TUCKER;
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Abstract: After being considered a "vestigial organelle" for many years, recent research has provided convincing evidence of the crucial importance of primary cilia in the development and function of multiple components of the central nervous system. Notably, robust numbers of cilia appear in embryonic development shortly after elaboration of axons in sensory DRG neurons and persist throughout postnatal stages of maturation. Primary cilia can be eliminated through inactivation of genes that encode proteins essential for the maintenance of primary cilia, such as *Ift88*. Loss of *Ift88* should result in a working model for a lack of cilia in sensory areas that largely control pain processing, without affecting other biological processes in the animal. Mice expressing a CRE recombinase integrated into the TrpV1 locus are used as a tool to specifically eliminate ciliary genes in nociceptive neurons. We have crossed the TrpV1:CRE driver to an inducible mutant that we have successfully used to eliminate primary cilia in neurons of an *Ift88* flox mutant. The mouse model is tested for a variety of standard behavioral phenotyping and nociceptive-testing assays for indications of initial deficits in sensory/pain processing. More advanced testing assesses the role that nociceptor cilia play in response to tissue injury, including models of nerve injury. These assays offer insight on whether primary cilia expressed by postnatal nociceptive neurons of the DRG modulate nociceptive signals arriving from the periphery and thereby modulate nociceptive signaling under normal physiological conditions and/or during pathophysiological states.

Disclosures: K.L. Lindros: None. **E.J. Bilsky:** None. **K.L. Tucker:** None.

Poster

525. Pain Models: Physiology

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Topic: D.02. Somatosensation: Pain

Support: NIH grant NS045594

NIH grant NS055860

Title: Glucocorticoid receptor expression is decreased in the dorsal root ganglia in a rat model of inflammatory low back pain

Authors: S. I. A. IBRAHIM, W. XIE, *J. A. STRONG, J.-M. ZHANG;
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Abstract: Low back pain can be caused by disorders of lumbar intervertebral discs and compression of nerve roots. Because these conditions include inflammation, local injection of anti-inflammatory corticosteroids is a common treatment. However, current medications fail to work in many patients. Therefore, there is a need to optimize the treatments. Clinically used steroids for back pain injections target the glucocorticoid receptor (GR). In addition, they can activate the mineralocorticoid receptor (MR) with significant potency, which has a pro-inflammatory role in the dorsal root ganglia (DRG) that may offset the anti-inflammatory effects of GR activation. Local inflammation of the L5 DRG with the immune activator zymosan was used as a model for inflammatory low back pain. Local DRG inflammation induces mechanical hypersensitivity, mechanical allodynia and cold allodynia in the hindpaw. In the current study, we first characterized the expression pattern of the GR receptor in normal and inflamed DRG. Then, we examined the effectiveness of a GR agonist with and without the MR antagonist in alleviating local DRG inflammation-induced mechanical hypersensitivity. Using immunohistochemistry, we found that GR expression is widely expressed in the majority of neuronal and non-neuronal cells in the DRG. Local inflammation, however, significantly decreased GR expression on postoperative day 1. Eplerenone, a selective MR antagonist, was locally administered to the inflamed L5 DRG alone or in combination with dexamethasone. Although dexamethasone alone reduced pain behaviors, the effectiveness was improved when combined with eplerenone. The ineffectiveness of the epidural steroid injections may be attributed to the reduced expression/activation of GR in the inflamed sensory ganglia as well as to concurrent activation of the MR.

Disclosures: **S.I.A. Ibrahim:** None. **W. Xie:** None. **J.A. Strong:** None. **J. Zhang:** None.

Poster

525. Pain Models: Physiology

Location: Halls B-H

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Topic: D.02. Somatosensation: Pain

Support: William And Ella Owens Foundation

American Cancer Society

Title: A novel method to study tumor-nerve interaction for oral cancer pain

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Abstract: Pain is often the presenting and the top ranked symptom in oral cancer patients, leading to significant reduction in quality of life. Unlike other cancers, pain in these patients is produced at the primary site of the tumor even when the tumor is still quite small in size suggesting that tumor cells control the activity of surrounding nociceptors. It is therefore crucial to understand the interaction of oral tumor cells with surrounding sensory nerves to delineate mechanisms by which oral cancer produces pain. The current study developed and validated a novel orthotopic in vivo tongue cancer model that mimics patient symptoms. Moreover, using this model, we have established a novel electrophysiology method that allows us to study tumor-nerve interactions at the site of primary tumor growth.
Injection of human oral squamous cell carcinoma cells (HSC2) into the tongue of athymic mice produces a well-differentiated tumor by day 6 post-inoculation. Control groups received normal human oral keratinocyte cells in the tongue. By day 9, tumor-bearing animals demonstrated patient reported symptoms such as pain during eating, ongoing pain and facial pain as measured by feeding behavior, conditioned placed preference test and Von Frey testing in the vibrissae respectively. We then, used this in vivo model to dissect out the tumor growing tongue or normal tongue and its associated lingual nerve to determine and characterize lingual nerve fibers and their firing responses in response to the tumor. We were able to successfully record increased nerve discharges upon mechanical stimulations and compare with normal tongues.
Our in vivo orthotopic tumor model is the first oral cancer pain model that reflects patient symptoms. We have now used this model to develop a tumor-tongue electrophysiology method that allows studying interactions of tumor and peripheral nerve fibers. Currently we are characterizing the different types of fibers with different stimulus modalities to determine the effect of oral tumor on sensory firing.

Disclosures: **S. Ruparel:** None. **M. Bendele:** None. **L. Chodroff:** None. **A. Wallace:** None. **V. Valenzuela:** None.

Poster

525. Pain Models: Physiology

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Topic: D.02. Somatosensation: Pain

Support: Fulbright Commision Romania

NIH AR057194

Title: Serotonin induces distinct types of responses in primary cultures from rat dorsal root ganglia

Authors: D. DOMOCOS[1], T. SELESCU[1], *E. E. CARSTENS[2,1], M. IODI CARSTENS[2], A. BABES[1];
[1]Anatomy, Physiol. and Biophysics, Univ. of Bucharest, Bucharest, Romania; [2]Neurobiology, Physiol. & Behavior, Univ. of California Davis, Davis, CA

Abstract: Serotonin (5-HT) is an important inflammatory mediator of both pain and itch. The mechanisms through which 5-HT activates primary sensory neurons are not completely established. We used calcium microfluorimetry and pharmacological tools to investigate the action of 5-HT in primary cultures of rat sensory neurons. Dorsal root ganglia (DRG) were dissected out from adult Wistar rats and dissociated neurons were cultured on glass coverslips. After 24 h, the cells were loaded with Calcium Green-1 AM and imaged while being chemically stimulated using a fast-exchange superfusion system. Based on their kinetics we classified the responses to 5-HT (50 μM) as transient, spiky and sustained. Most of the 5-HT-sensitive neurons had only transient responses and were positive for Isolectin-B4 binding. The transient responses were also elicited by the 5-HT3 agonist SR 57227 (1-10 μM) and were completely inhibited by the 5-HT3 antagonist granisetron (1 μM). The 5-HT4 agonists cisapride and tegaserod maleate activated a sub-population of neurons different from the one responding to 5-HT. On the other hand, the non-selective agonist 5-carboxamidotryptamine (5-CT, 50 μM) induced only spiky responses, solely in the neurons responding in the same manner to 5-HT. Recently reported activators or inhibitors of 5-HT receptors from mouse DRG neurons, LY344864 (a 5-HT1F agonist), LP44 (a 5-HT7 agonist) and SB269970 (a 5-HT7 antagonist) had no effects on rat DRG neurons. Nominally calcium-free solutions abolished the transient and spiky components as did the co-application of 5-HT with lanthanum, a non-selective calcium channel (Ca_v) blocker. The

L-, N- and T-type Ca_v antagonists nifedipine, cilnidipine and mibefradil respectively, had no inhibitory action on the 5-HT-elicited responses. Ruthenium red, a broad range blocker of transient receptor potential (TRP) ion channels had either no effect, or enhanced the spiky responses. All the responses to 5-HT were abolished when sodium ions were replaced with N-methyl-D-glucamine. Activation of spiky responding neurons by 5-HT was simultaneously recorded using calcium imaging and patch clamp. In voltage clamp mode, at a holding potential of -80 mV, 5-HT activated only transient currents (inhibited by granisetron), while in current clamp mode it triggered bursts of action potentials accompanied by intracellular calcium increase. Taken together, our results show that 5-HT3 receptors mediate the transient responses to 5-HT, while the more sustained calcium entry accompanies action potentials following activation of not yet identified 5-HT receptors, also sensitive to 5-CT.

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Poster

525. Pain Models: Physiology

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Program#/Poster#: 525.23/SS10

Topic: D.02. Somatosensation: Pain

Title: Mechanisms underlying the excitation of rat sensory neurons via metabotropic 5-HT receptors

Authors: *E. GANTUMUR, I. SALZER, A. YOUSUF, S. BOEHM;
Med. Univ. of Vienna, Wien, Austria

Abstract: Serotonin (5-HT) is an inflammatory mediator and involved in pain sensation. Ionotropic 5-HT_3 receptors of dorsal root ganglion (DRG) neurons are thought to mediate this effect. However, the role of metabotropic 5-HT receptors remained controversial. Here, the contribution of metabotropic 5-HT receptors and their functional interactions with Kv7, TTX-resistant Na^+ channels, TRPV1 and Ca^{2+}-activated Cl^- channels (CaCCs) were investigated. Using the perforated and whole-cell patch clamp technique in voltage and current clamp mode on primary cultures of rat DRG neurons, effects of 5-HT receptor ligands on membrane potential and currents through Kv7, TTX-resistant Na^+ channels, TRPV1 and Ca^{2+}-activated Cl^- channels were investigated. 5-HT depolarized DRG neurons and increased their excitability. This effect was not altered by the 5-HT_3 receptor antagonist tropisetron, but reduced by the 5-HT_2 receptor antagonist ritanserin. Moreover, this increased excitation of DRG neurons by 5-HT was inhibited by TRPV1 antagonist capsazepine and CaCC blocker CaCC-Inh A01. Furthermore, this 5-HT-

induced excitation was inhibited by the 5-HT_{2C} receptor-specific antagonist RS102221 hydrochloride, but not by the 5-HT_{2A} receptor-specific antagonist 4F4PP oxalate or the 5-HT_{2B} receptor-specific antagonist SB204741. Currents through Kv7 channels and TTX-resistant Na^+ channels of DRG neurons were not affected by 5-HT. In contrast, 5-HT enhanced currents through TRPV1 channels in DRG neurons. This increase of TRPV1 current was inhibited by the non-selective 5-HT_2 receptor antagonist ritanserin as well as the specific antagonists of 5-HT_{2A} (4F4PP oxalate), 5-HT_{2B} (SB204741), and 5-HT_{2C} (RS102221 hydrochloride) receptors. Additionally, 5-HT also induced currents through CaCCs which were inhibited by the CaCC blocker CaCC-Inh A01 and the 5-HT_{2C} receptor-specific antagonist RS102221 hydrochloride, but not by the 5-HT_{2A} (4F4PP oxalate) or the 5-HT_{2B} (SB204741) receptor-specific antagonists. These results show that 5-HT enhances the excitability of DRG neurons via 5-HT_2 receptors with a major contribution of 5-HT_{2C} which simultaneously mediates a potentiation of TRPV1 channels and an activation of CaCCs.

Disclosures: E. Gantumur: None. **I. Salzer:** None. **A. Yousuf:** None. **S. Boehm:** None.

Poster

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Title: Identification of metabolite-sensing muscle afferents *In vivo* using GCaMP6s.

Authors: *K. M. SMITH-EDWARDS[1], A. R. LIGHT[2], C. J. WOODBURY[1];
[1]Zoology & Physiol., Univ. of Wyoming, Laramie, WY; [2]Univ. of Utah, Salt Lake City, UT

Abstract: Although many chronic pain conditions are musculoskeletal in nature, the sensory inflow from muscles under different metabolic states is poorly understood. Recently, two populations of muscle afferents were shown to respond to different concentrations of muscle metabolites in reduced preparations (Light et al., J Neurophys 100: 1184, 2008; Jankowski et al., J Neurophys 109 : 2374, 2013); metaboreceptors responded best to lower levels found in muscle following moderate exercise and may contribute to the sense of fatigue, whereas metabonociceptors responded best to higher levels found after strenuous and/or ischemic

contractions and may contribute to pain (Pollak et al., Exp Physiol 99:368, 2014). To better understand the properties of metabolite-sensing muscle afferents in vivo we imaged population responses of these cells in GCaMP mice. Briefly, mice with GCaMP6s expression in all cells were anesthetized, L3-4 DRGs exposed, and DRG cells imaged while infusing the gastrocnemius with different metabolite solutions [control: 300nM ATP, 1mM lactate, pH 7.4; low: 1μM ATP, 15mM lactate, pH 7.0; high: 5μM ATP, 50mM lactate, pH 6.6; N=6). To date, 40 DRG cells that responded to infusions have been characterized. A few (n=6) responded equally well to control and metabolite infusions and were presumably chemically-insensitive mechanoreceptors. However, most in our sample (n=34) responded robustly to elevated metabolite levels and could be divided into two subpopulations. Half responded best to high metabolite levels (17.03 ± 3.35 $\Delta F/F$, n=17, $p<0.01$, ANOVA w/ Tukey's) and were presumably metabonociceptors, whereas the rest responded best to low metabolite levels (16.61 ± 2.68 $\Delta F/F$, n=17, $p<0.05$) and thus identified as metaboreceptors. Interestingly, the cell bodies of most metaboreceptors were small (76% ≤ 27 μm) whereas the majority of metabonociceptors had medium-to-large diameter somata (60% between 28-43 μm), further suggesting these are distinct populations. While some metaboreceptors also displayed sensitivity to mechanical (3/17) and/or heat stimuli (5/17), most were found to respond only to metabolites (10/17). Overall, these findings confirm two populations of metabolite-sensing muscle afferents in vivo under normal conditions and suggest that the majority of metaboreceptors exhibit fine-diameter afferent fibers that are selective for metabolites. Further studies using GCaMP to examine plasticity of these afferents in vivo may provide valuable insight into the development of chronic musculoskeletal pain.

Disclosures: K.M. Smith-Edwards: None. **A.R. Light:** None. **C.J. Woodbury:** None.

Poster

525. Pain Models: Physiology

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Topic: D.02. Somatosensation: Pain

Support: NIH Grant DE018661

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Title: Operant tests assessing rat orofacial mechanical pain induced by systemic administration of oxaliplatin and local injection of Complete Freund's Aadjuvant into temporomandibular joints

Authors: *V. VIATCHENKO-KARPINSKI, F. EROL, J. LING, J. GU;
Anesthesiol., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Chronic orofacial pain conditions are important clinical problems that are poorly treated. Two main causes of chronic orofacial pain states are cranial nerve neuropathy and orofacial tissue inflammation. Common symptoms associated with these pathological conditions are mechanical allodynia and hyperalgesia. In the present study, the chemotherapy drug oxaliplatin, which is known to be able to induce peripheral neuropathy, was systemically administered to rats to induce neuropathic pain. Complete Freund's Adjuvant (CFA) was locally injected into temporomandibular joints (TMJ) to induce inflammatory pain of TMJ. We assessed orofacial mechanical pain in these two animal models using the orofacial operant test. In both pathological pain models, the operant test showed significant changes in operant behaviors in the presence of mechanical stimulation, which indicated orofacial mechanical allodynia/hyperalgesia in these animals. We determined whether the hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels may be involved in the orofacial mechanical allodynia/hyperalgesia by testing ZD 7288, a blocker of HCN channels, on orofacial operant behaviors. Administration of ZD 7288 at the dose of 25 μg/kg (*i.p.* injection) showed no significant improvement of orofacial operant behaviors in oxaliplatin-induced orofacial pain model. We determined whether mechanical allodynia/hyperalgesia induced by oxaliplatin could be alleviated by retigabine, a KCNQ2/3 channel potentiator. We found that retigabine at the dose of 2 mg/kg (*i.p.* injection) significantly improved orofacial operant behaviors. KCNQ2/3 channels may provide an effective therapeutic target for the treatment of chronic orofacial pain manifested with mechanical allodynia/hyperalgesia.

Disclosures: V. Viatchenko-Karpinski: None. **F. Erol:** None. **J. Ling:** None. **J. Gu:** None.

Poster

525. Pain Models: Physiology

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Topic: D.02. Somatosensation: Pain

Title: EPAC is responsive for the exacerbation of the injury-induced pain hypersensitivity after the second surgery

Authors: *M. MATSUDA, F. AMAYA;
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Abstract: Background
Activation of cAMP sensor protein EPAC in the DRG is associated with the development of chronic pain. Injury-induced pain hypersensitivity after the second surgery is greater than initial one, even the baseline sensitivity prior to the succeeding injury has been restored. In the present

study, we investigated the role of EPAC signaling in the DRG for the exacerbation of the injury-induced pain hypersensitivity after the second surgery.
Methods
Male SD rats (200-250g) were used for the experiments. All experimental procedures were approved by the animal ethics committee of the Kyoto Prefectural University of Medicine. Plantar incision model was used for a model of surgical injury. Initial and succeeding incisions were performed on left plantar with 14days intervals. Withdrawal threshold against mechanical stimulation by von Frey hair was analyzed up to 10days after each incision. To see the effect of EPAC inhibitor ESI-09 on pain hypersensitivity after the incision, animals were divided into two groups. ESI1 group received intraplantar injection of ESI-09 (25μg) 2hours prior to the first surgery. ESI2 group received intraplantar injection of ESI-09 (25μg) 2hours prior to the second surgery. Control group received same amount of vehicle.
Results
Plantar incision induced reduction of mechanical threshold against von Frey stimulation, indicating injury-induced pain hypersensitivity. Pain hypersensitivity was detected up to 5days after the first surgery. Succeeding injury induced longer pain hypersensitivity continuing for 10days. Mechanical threshold at 7days after the second surgery was significantly lower compared to the first surgery. ESI-09 inhibited prolonged pain hypersensitivity observed after the second surgery, but had no effect on the pain hypersensitivity after the first surgery.
Conclusion
Second surgery induces prolongation of the injury-induced pain hypersensitivity compared to the first surgery. EPAC signaling involves in the pain exacerbation after the second surgery.

Disclosures: **M. Matsuda:** None. **F. Amaya:** None.

Poster

525. Pain Models: Physiology

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Topic: D.02. Somatosensation: Pain

Support: Rita Allen Foundation/American Pain Society

IASP Early Career Grant

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Title: Prevention of muscle IL1β upregulation blocks ischemia/reperfusion injury-induced muscle afferent sensitization and pain-related behaviors

Authors: *J. L. ROSS, J. E. LAMB, L. F. QUEME, B. KATRAGADDA, Z. K. FORD, M. P. JANKOWSKI;
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Abstract: Musculoskeletal pain is a widely experienced, yet relatively understudied, clinical issue. Complications with peripheral circulation have been increasingly suggested as an underlying cause of muscle pain in multiple disease states, including sickle cell anemia and peripheral vascular disease. In our model of transient ischemia with reperfusion injury (I/R) in male Swiss Webster mice, we previously showed altered chemosensitivity and mechanical thresholds in single group III and IV muscle afferents as assessed with an *ex vivo* muscle-nerve-dorsal root ganglion (DRG)-spinal cord recording preparation. Primary afferent sensitization correlated with increased spontaneous (guarding) and evoked (von Frey mechanical stimulation) pain-related behaviors as well as decreased muscle strength and voluntary activity *in vivo*. These alterations in pain-like behaviors and sensory neuron responsiveness appeared to be due to increased interleukin 1β (IL1β) in the injured muscles acting at the upregulated interleukin 1 receptor (IL1r1) within the DRGs, as an afferent-specific knockdown of IL1r1 inhibited I/R-induced changes in afferent response properties and animal behaviors. In this study, we have used two additional strategies to effectively block the I/R-induced increase in muscle IL1β. Here we show that voluntary wheel running for two days prior to I/R not only prevented this IL1β enhancement, but also ablated the subsequent development of ischemic myalgia-like behaviors (increased paw guarding and reduced mechanical threshold, grip strength, and voluntary activity). In addition, systemic injection of the IL1 receptor antagonist (IL1RA), which would also block the effects of IL1β during I/R, was similarly found to prevent injury-induced upregulation of IL1r1 within the DRGs, and recapitulated most of the preventative effects of exercise or nerve-specific IL1r1 knockdown on I/R-related behaviors. IL1RA-treated animals, however, still experienced an I/R-induced grip strength deficit, suggesting that systemic inhibition of IL1β may potentially have other effects on muscle repair and/or function. Nevertheless, IL1RA was still found to block I/R-induced changes in afferent mechanical thresholds, chemosensitive muscle afferent prevalence, and gene expression in the affected DRGs. Altogether, these data strengthen the evidence that transient ischemia and reperfusion injury sensitizes group III and IV muscle afferents via increased IL1β in the muscles, which leads to the development of ischemic myalgia. This work may also provide insight into potential therapies for the prevention of persistent muscle pain initiated by an ischemic insult.

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Poster

525. Pain Models: Physiology

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Topic: D.02. Somatosensation: Pain

Support: JSPS KAKENHI 15K08673

Title: Inward current and spontaneous excitatory transmission enhancement produced by orexin B in adult rat spinal substantia gelatinosa neurons

Authors: C. WANG, T. FUJITA, T. YU, R. HIRAO, N. MAGORI, R. SUZUKI, *E. KUMAMOTO;
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Abstract: There is much evidence showing that oxytocin or orexin (hypocretin) originating from the hypothalamus inhibits nociceptive transmission from the periphery in the spinal dorsal horn. We have previously reported that oxytocin (0.5 μM) produces an inward current at a holding potential of -70 mV in 67 % of the spinal dorsal horn lamina II (substantia gelatinosa; SG) neurons examined without a change in glutamatergic spontaneous excitatory transmission. The SG neurons play a pivotal role in regulating nociceptive transmission from the periphery. An action of orexin on synaptic transmission in adult rat SG neurons has not yet been examined fully. In order to know cellular mechanisms for antinociception produced by orexin B (hypocretin 2), we examined its effect on holding current and spontaneous excitatory transmission at -70 mV by applying the blind whole-cell patch-clamp technique to SG neurons of adult rat spinal cord slices. In 17 % of the SG neurons examined (n = 129), orexin B (0.05 μM) superfused for 2 min produced an inward current which was not accompanied by a change in spontaneous excitatory transmission. On the other hand, 19 % of the SG neurons did not alter holding currents while exhibiting an increase in the frequency of spontaneous excitatory postsynaptic current (sEPSC). Both of the inward current and sEPSC frequency increase were produced in 27 % of the SG neurons. The sEPSC frequency increase subsided within 10 min after washout of orexin B. The inward current had the averaged peak amplitude of 6.8 ± 0.3 pA (n = 57) and sEPSC frequency around 2 min after the onset of orexin B superfusion averaged to be 188 ± 8 % (n = 59) of that before its superfusion with no change in its amplitude. Remaining neurons (n = 48; 37 % of the neurons) did not respond to orexin B. The inward current and sEPSC frequency increase were repeated at a time interval of 20 min. The orexin B activities were concentration-dependent; half-maximal effective concentration values for the inward current and sEPSC frequency increase were 0.020 and 0.066 μM, respectively. These results indicate that orexin B produces a membrane depolarization in SG neurons and increases the spontaneous release of L-glutamate to SG neurons from nerve terminals; the former action is

similar to that of oxytocin. Such orexin B actions could contribute to its antinociceptive action in the spinal dorsal horn.

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Poster

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Topic: D.02. Somatosensation: Pain

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Title: Dissecting protein kinase C-sensitive adenylyl cyclase signaling complexes in mouse sensory neurons with affinity purification mass spectrometry

Authors: C. ESANCY, B. K. DRAGOO, R. GEGUCHADZE, *D. C. MOLLIVER;
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Abstract: Gs-coupled metabotropic receptors play a significant role in the sensitization of nociceptors in response to inflammatory insult or injury, by activating the adenylyl cyclase (AC)-cAMP-protein kinase A (PKA) pathway. Conversely, ACs are inhibited by Gi/o-coupled receptors, which is one mechanism underlying analgesia provided by opiates. Type II AC isoforms are uniquely regulated in that they can be activated by protein kinase C (PKC) as well as Gs, making them potential integrators of signaling through multiple second messenger pathways usually considered to be distinct. Coincident activation of Gq/11-coupled receptors (leading to activation of PKC) and Gs-coupled receptors is likely to occur in response to peripheral injury or insult. Furthermore, Type II ACs are insensitive to inhibition by Gi/o-coupled receptors, suggesting the possibility that these AC isoforms, if expressed in primary afferent nociceptors, could contribute to persistent pain recalcitrant to descending inhibition or exogenous opiates. Therefore, we examined the distribution of Type II ACs ADCY2, ADCY4 and ADCY7 in sensory neurons of the dorsal root ganglion (DRG) from adult male mice. Real-time PCR revealed mRNA for all 3 isoforms in whole DRG (ADCY2 > ADCY7 > ADCY4). RNA levels did not change significantly 3 days after inflammation induced by hindpaw injection of complete Freund's adjuvant or 7 days after sciatic nerve crush. Analysis of mRNA levels in neuronally-enriched and glial-enriched DRG fractions by endpoint RT-PCR indicated expression

of ADCY2 and 7 mRNA in DRG neurons. Immunohistochemistry revealed intense staining of small-diameter neurons for AC2, the majority of which were also positive for TRPV1. We next used affinity purification mass spectrometry to identify binding partners of AC2, using antibodies to the endogenous protein. Two dimensional difference gel electrophoresis (2D DiGE) was analyzed with DeCyder 7 software to identify any changes in binding partners in response to nerve injury. Spots of interest on 2D gels were picked, fragmented with trypsin and analyzed by MALDI ToF peptide mass fingerprinting to characterize protein complexes associated with AC2 in DRG from healthy and nerve-injured mice.

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Poster

525. Pain Models: Physiology

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Topic: D.02. Somatosensation: Pain

Support: NIH grant R01 DA033059

Title: A rapid method for selective enrichment of Nav1.8-containing sensory neuron synaptosomes from the rat spinal cord

Authors: *J. A. MCROBERTS, H. S. ENNES, J. C. MARVIZON;
Dept Med., UCLA, Los Angeles, CA

Abstract: Much of what is known about the presynaptic terminals of sensory neurons in the spinal cord has been inferred from studies of dorsal root ganglia cell bodies, measurement of neurotransmitter release, or co-localization of antibodies in spinal cord sections. The objective of this study was to selectively enrich synaptosomes prepared from the spinal cord of rats for those arising from synaptic terminals of small diameter sensory neurons involved in pain transmission. An antibody recognizing an extracellular epitope of the voltage gated sodium channel, Nav1.8 (Alomone) was covalently crosslinked to amine-derivatized MagnaBind beads (Thermo Sci) using BS^3 as a crosslinker. After quenching the reaction with Tris, the beads were washed with PBS and blocked with a protein-free blocking buffer. Non-derivatized beads were prepared identically except that the antibody was left out of the reaction. For rapid isolation of synaptosomes, spinal cord tissue was homogenized with a Dounce tissue grinder in Syn-PER reagent (Thermo Sci). The homogenate was centrifuged at 1200 *g* for 10 min and the supernatant (crude extract) centrifuged at 15,000 *g* for 20 min. A sample of the supernatant (cytosol) was

collected for analysis and the pellet (crude synaptosomes) resuspended and preabsorbed with the non-derivatized beads for 1 hr. The beads were removed and the synaptosomes added to the Nav1.8-derivatized beads for 2 hr. After washing, the affinity enriched synaptosomes were eluted from the MagnaBind beads with buffer containing 1% SDS. All fractions were subjected to SDS gel electrophoresis and western blots were probed with antibodies to synapsin-1, Nav1.8, TrpV1, and Cav2.2. The density of each band was measured and normalized to that of the crude extract. As shown in the table, the crude synaptsome preparation was enriched in all 4 proteins, however the Nav1.8 affinity purified synaptosomes were selectively enriched in the heat and acid sensing channel, TrpV1, and N-type calcium channel α1 subunit, Cav2.2, as well as Nav1.8. This preparation may be useful in identifying changes in the phosphorylation and protein composition of sensory neuron terminals in the spinal cord.

	Synapsin-1	Nav1.8	TrpV1	Cav2.2
crude extract	1.00	1.00	1.00	1.00
cytosol	0.68	0.52	0.23	0.06
crude synaptosomes	1.57	2.26	6.73	1.68
Nav1.8 enriched synaptosomes	2.22	4.94	14.14	4.22

Disclosures: J.A. McRoberts: None. **H.S. Ennes:** None. **J.C. Marvizon:** None.

Poster

526. Somatosensory Cortical Circuits: Blue Brain Project

Location: Halls B-H

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Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano

Title: Topological analysis of digital reconstructions of neocortical microcircuits

Authors: ***M. NOLTE**[1], M. W. REIMANN[1], P. DŁOTKO[3], K. HESS[2], R. LEVI[4], M. SCOLAMIERO[2], K. TURNER[2], E. MULLER[1], H. MARKRAM[1];
[1]Blue Brain Project, EPFL, Geneva, Switzerland; [2]Lab. for Topology and Neurosci., EPFL, Lausanne, Switzerland; [3]Geometrica, Inria, Saclay, France; [4]Univ. of Aberdeen, Aberdeen, United Kingdom

Abstract: A first draft digital reconstruction and simulation of the somatosensory cortical microcircuit of a two-week-old rat was recently published. Using this resource, we explored whether methods from algebraic topology can provide a novel and useful perspective on the structural and functional organization of the microcircuit. Structural topological analysis revealed that directed graphs representing the connectivity between neurons are significantly different from random graphs even when distance dependence of connectivity is taken into account. Further, there exist an enormous number of simplicial complexes of different dimensions representing all-to-all connections within different sets of neurons, the most extreme motif of neuronal clustering reported so far in the brain. Functional topological analysis based on data from simulations confirmed the interest of the new approach to studying the relationship between the structure of the connectome and its emergent functions.

Disclosures: **M. Nolte:** None. **M.W. Reimann:** None. **P. Dłotko:** None. **K. Hess:** None. **R. Levi:** None. **M. Scolamiero:** None. **K. Turner:** None. **E. Muller:** None. **H. Markram:** None.

Poster

526. Somatosensory Cortical Circuits: Blue Brain Project

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Title: Systematic integration of literature-derived knowledge into data-driven large-scale neuronal modeling workflows

Authors: *C. O'REILLY, E. IAVARONE, S. HILL;
Blue Brain Project, École Polytechnique Fédérale De Lausanne, Geneva, Switzerland

Abstract: Building large-scale realistic models of neuronal circuits such as those being designed in the context of the Blue Brain Project requires setting values to a large number of parameters. Previously reported values must therefore be systematically extracted from the literature, in some cases to be directly included in the model (e.g., when no experimental data are available) or for validation purposes. This process requires an extensive and time consuming review of the literature. Further, without appropriate curation methodology, the outcome of this work is generally poorly reusable between projects. Moreover, without a systematic way to include these values into models, the traceability of their origin can be easily lost. To address these issues, we have developed a series of open-source tools to systematically and collaboratively curate the relevant literature (NeuroCurator[1,2]), share this information in annotation corpora, and systematically query corpora (thanks to links with ontological terms) to feed appropriate values to models (MetaModeler[3]). Inserted values are associated with unique IDs, which allow to find the exact source of populated values (i.e., not only an unambiguous reference to the original publication but also the precise place where this value is reported in the original document). This software pipeline has been designed in Python with user-friendly Qt-based graphical user interfaces, as well as back-end APIs allowing manipulating corpora programmatically, e.g., through Jupyter (IPython) notebooks. We demonstrate the usefulness of this approach by showing its application in modeling the thalamocortical loop in the context of the Blue Brain Project. In future work, we want to better integrate these software with other existing tools, both annotation producers (e.g., annotation interfaces such as Hypothes.is[4] or B2Note[5] as well as text-mining applications such as Sherlok[6]) and consumers (e.g., web interfaces such as Knowledge-Space[7]).

[1] O'Reilly, C., Iavarone, E., & Hill, S. NeuroCurator: Collaborative creation of sharable corpus for data-driven computational modeling, Keystone Symposia, State of the Brain, Alpbach, Austria, May 22-26, 2016.

[2] https://github.com/christian-oreilly/neurocurator

[3] https://github.com/christian-oreilly/metamodeler

[4] https://hypothes.is/

[5] http://b2note.bsc.es/devel/

[6] Richardet, R, Chappelier, JC, Tripathy, S, & Hill, S Agile text mining with Sherlok, 2015 IEEE International Conference on Big Data, Santa Clara, CA, October 29-November 1, 2015, 1479-1484.

[7] http://knowledge-space.org/

Disclosures: C. O'Reilly: None. **E. Iavarone:** None. **S. Hill:** None.

Poster

526. Somatosensory Cortical Circuits: Blue Brain Project

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Title: Unraveling network structure of neocortical microcircuitry - hubs, motifs, small-world and e/i ratio.

Authors: *E. GAL[1], A. GLOBERSON[3], M. LONDON[2], S. RAMASWAMY[4], M. REIMANN[4], E. MULLER[4], H. MARKRAM[4], I. SEGEV[2];
[1]Edmond and Lily Safra Ctr. for Brain Sci., [2]Dept. of Neurobio., The Hebrew Univ., Jerusalem, Israel; [3]The Blavatnik Sch. of Computer Sci., Tel Aviv Univ., Tel Aviv, Israel; [4]Blue Brain Project, École Polytechnique Fédérale de Lausanne (EPFL) Biotech Campus Geneva, Lausanne, Switzerland

Abstract: Uncovering hidden regularities and topological structures of cortical microcircuitry is vital for understanding neural computation. Recent experimentally-constrained dense in silico reconstructions of ~0.3mm^3 of rat somatosensory neocortex, consisting of ~31,000 neurons and ~40 million synapses, have provided an unprecedented opportunity to analyze microcircuitry at the synaptic-level. We identified multiple wiring specificities, distinct for excitatory (E) and inhibitory (I) populations. The number of E and I synapses/cell varied across neurons, yet excitatory neurons maintained relatively constant E/I ratio. The microcircuit contained highly connected hub-neurons, belonging to a small subset of cell types; these hubs are strongly interconnected among themselves, forming cell-type-specific rich-clubs. Certain three-neuron motifs were overrepresented, matching recent experimental results. Global small-world topology was observed, with an average of 2.5 synapses separating any two neurons. By unraveling the topological backbone of a dense neocortical microcircuit, this study provides several experimentally testable predictions and a systematic approach to interpret micro-connectomics "big data".

Disclosures: **E. Gal:** None. **A. Globerson:** None. **M. London:** None. **S. Ramaswamy:** None. **M. Reimann:** None. **E. Muller:** None. **H. Markram:** None. **I. Segev:** None.

Poster

526. Somatosensory Cortical Circuits: Blue Brain Project

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Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano

Title: BluePyOpt: Leveraging Python and the cloud to optimise models to neuroscience data

Authors: ***J.-D. COURCOL**[1], W. VAN GEIT*[1], M. GEVAERT[1], G. CHINDEMI[1], C. ROESSERT[1], E. MULLER[1], F. SCHUERMANN[1], I. SEGEV[2], H. MARKRAM[1];
[1]Campus Biotech, EPFL - Blue Brain Project, Geneva, Switzerland; [2]Dept. of Neurobio., Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: At many scales in neuroscience, appropriate mathematical models often take the form of complex dynamical systems. Constraining such models to the multitude of available experimental data is a global nonlinear optimisation problem with a complex fitness landscape, generally requiring heuristic techniques to find suitable approximate solutions. Stochastic optimisation approaches, such as evolutionary algorithms, have been shown to be effective, but often the setting up of such optimisations is non-trivial, requiring domain-specific expertise. Here we describe BluePyOpt, an extensible open-source Python-based framework targeted at the broad neuroscience community to simplify the development of data-driven model parameter optimisations. The versatility of the BluePyOpt framework is demonstrated by working through three representative neuroscience specific use cases: point-neuron and morphologically detailed neuron models, and a calcium-based model of synaptic plasticity. Further, BluePyOpt provides methods for setting up both small- and large-scale optimisations on a variety of platforms, ranging from laptops to Linux clusters and cloud-based compute infrastructures. BluePyOpt is intended as a resource for the community, and as been deployed on public resources such as the

Human Brain Project Brain Simulation Platform (https://collab.humanbrainproject.eu), and the Neuroscience Gateway (http://www.nsgportal.org).

Disclosures: **J. Courcol:** None. **W. Van Geit*:** None. **M. Gevaert:** None. **G. Chindemi:** None. **C. Roessert:** None. **E. Muller:** None. **F. Schuermann:** None. **I. Segev:** None. **H. Markram:** None.

Poster

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Support: European Union Seventh Framework Program (FP7/2007-2013) under grant agreement no. 604102 (HBP)

Title: Unique membrane and enhanced computations in human neocortical neurons

Authors: ***G. EYAL**[1], M. B. VERHOOG[3], G. TESTA-SILVA[3], Y. DEITCHER[2], J. C. LODDER[3], R. BENAVIDES-PICCIONE[4], J. MORALES[5], J. DEFELIPE[4], C. P. J. DE KOCK[3], H. D. MANSVELDER[3], I. SEGEV[1,2];
[1]Dept. of Neurobio., [2]Edmond and Lily Safra Ctr. for Brain Sci., the Hebrew Univ. of Jerusalem, Jerusalem, Israel; [3]Dept. of Integrative Neurophysiology, Ctr. for Neurogenomics and Cognitive Res., VU Univ., Amsterdam, Netherlands; [4]Inst. Cajal (CSIC), and Lab. Cajal de Circuitos Corticales (CTB), [5]Escuela Técnica Superior de Ingenieros Informáticos, Univ. Politécnica de Madrid, Madrid, Spain

Abstract: Humans' advanced cognitive capabilities are often attributed to our recently evolved neocortex. However, we do not know whether neurons composing the human neocortex themselves possess unique biophysical or computational capabilities. Combining rare physiological and anatomical data with modeling approach, we find that layer 2/3 pyramidal cells in human temporal cortex (HL2/3 PCs) are exceptional in three major ways. First, the cell membranes have a surprisingly low specific capacitance (C_m = ~0.5 μF/cm^2), which promotes excitability. Second, they have disproportionally elongated distal basal dendrites, which receive powerful (AMPA + NMDA) synaptic input, providing the cells with a large number of semi-independent nonlinear functional subunits. Third, the cells generate axonal Na^+ spikes upon activation of 80-120 excitatory synapses (out of 15,000-30,000), endowing them with a huge combinatorial input/output repertoire. Our study provides the first-ever comprehensive model of

any human neuron and demonstrates the biophysical and computational uniqueness of human cortical neurons.

Disclosures: **G. Eyal:** None. **M.B. Verhoog:** None. **G. Testa-Silva:** None. **Y. Deitcher:** None. **J.C. Lodder:** None. **R. Benavides-Piccione:** None. **J. Morales:** None. **J. DeFelipe:** None. **C.P.J. de Kock:** None. **H.D. Mansvelder:** None. **I. Segev:** None.

Poster

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Title: Extracellular calcium influences on long-term plasticity

Authors: ***R. PERIN**[1], G. CHINDEMI[2], E. MUELLER[2], H. MARKRAM[2];
[1]Brain Mind Institute, EPFL, Lausanne, Switzerland; [2]EPFL, Lausanne, Switzerland

Abstract: Calcium is an essential ion involved in determining how neuronal activity impacts long-term synaptic plasticity. Here we studied the effects of varying extracellular calcium concentration on long-term potentiation and depression in thick-tufted layer 5 pyramidal neurons of the somatosensory cortex of young rats (postnatal days 14 to 18). Patch-clamped neurons in acute brain slices *in vitro* maintained in extracellular calcium concentrations ranging from 1 mM to 2 mM were stimulated with either long-term depression or facilitation protocols. We observed that in certain cases the same protocol applied under different calcium concentrations produced very different effects. These results suggest that synaptic plasticity *in vivo* would be influenced by calcium concentration changes found between sleep and wakefulness and may also be affected by network states, which may cause short-term changes in the levels of calcium.

Disclosures: **R. Perin:** A. Employment/Salary (full or part-time): EPFL. **G. Chindemi:** A. Employment/Salary (full or part-time): EPFL. Other; The EPFL Blue Brain Project, The ETH Board Funding to the Blue Brain Project, European Union Seventh Framework Program (FP7/2007-2013) under grant agreement no. 604102 (HBP), Calculations were performed on the

EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano. **E. Mueller:** A. Employment/Salary (full or part-time): EPFL. Other; The EPFL Blue Brain Project, The ETH Board Funding to the Blue Brain Project, European Union Seventh Framework Project (FP7/2007-2013) under grant agreement no. 604102 (HBP), Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano. **H. Markram:** A. Employment/Salary (full or part-time): EPFL. Other; The EPFL Blue Brain Project, The ETH Board Funding to the Blue Brain Project, European Union Seventh Framework Program (FP7/2007-2013) under grant agreement no. 604102 (HBP), Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano.

Poster

526. Somatosensory Cortical Circuits: Blue Brain Project

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Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano

Title: The 2016.10 release of the rat somatosensory microcircuit

Authors: *E. B. MULLER[1], S. RAMASWAMY*[1], M. REIMANN*[1], O. AMSALEM[2], G. CHINDEMI[1], J.-D. COURCOL[1], A. DEVRESSE[1], J. DYNES[1], M. GEVAERT[1], K. HESS[1], L. KANARI[1], D. KELLER[1], Y. KIM[1], J. G. KING[1], Z. KISVÁRDAY[3], J. MEYSTRE[4], T. NEWTON[1], R. PERIN[4], J. RAHMON[1], C. ROESSERT[1], Y. SHI[1], J. SHILLCOCK[1], M. SRIVASTAVA[3], W. VAN GEIT[1], Y. WANG[5,6], J. DEFELIPE[7,8], S. HILL[1], F. SCHUERMANN[1], I. SEGEV[2], H. MARKRAM[1];
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Med. Ctr., Tufts Univ., Boston, MA; [6]Sch. of Optometry and Opthalmology, Wenzhou Med. Col., Wenzhou, China; [7]Lab. Cajal de Circuitos Corticales, Univ. Politecnica de Madrid, Madrid, Spain; [8]Inst. Cajal, Madrid, Spain

Abstract: The Blue Brain Project has developed a data-driven process for the digital reconstruction of juvenile rat somatosensory cortex by integrating biological data on its cellular and synaptic anatomy and physiology. The resulting microcircuit reconstruction was released in October, 2015 and was found to be broadly consistent with the state of knowledge on the structure and function of neocortical microcircuitry. For the October, 2016 (2016.10) release of the microcircuit, a series of updates and refinements have been made, including morphological classification of pyramidal neurons based on newly developed topological methods, axonal morphologies based on in vivo axonal reconstructions and synthesis approaches, models of synaptic transmission accounting for multi-vesicular release and long-tailed synaptic physiology distributions, neuron model parameterizations for each electrical type were optimized for each morphological type, structural representation of glia, addition of gap junctions between inhibitory neurons, and addition of synthesized thalamo-cortical axonal projections. We assessed quantitative differences compared to the previous version of the reconstruction across a suite of anatomical and physiological validations at the synaptic, cellular and network levels, and relate these differences to the underlying refinements. As with previous releases, the 2016.10 microcircuit release is available as a resource to the community through the NMC portal (https://bbp.epfl.ch/nmc-portal), and the Human Brain Project Brain Simulation Platform (https://collab.humanbrainproject.eu).

Disclosures: **E.B. Muller:** None. **S. Ramaswamy*:** None. **M. Reimann*:** None. **O. Amsalem:** None. **G. Chindemi:** None. **J. Courcol:** None. **A. Devresse:** None. **J. Dynes:** None. **M. Gevaert:** None. **K. Hess:** None. **L. Kanari:** None. **D. Keller:** None. **Y. Kim:** None. **J.G. King:** None. **Z. Kisvárday:** None. **J. Meystre:** None. **T. Newton:** None. **R. Perin:** None. **J. Rahmon:** None. **C. Roessert:** None. **Y. Shi:** None. **J. Shillcock:** None. **M. Srivastava:** None. **W. Van Geit:** None. **Y. Wang:** None. **J. Defelipe:** None. **S. Hill:** None. **F. Schuermann:** None. **I. Segev:** None. **H. Markram:** None.

Poster

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Title: A somatotopic distribution of physiological, morphological, and connectivity properties in the ventral posterolateral nucleus

Authors: *J. YI[1], E. IAVARONE[2], C. O'REILLY[2], R. PERIN[1], H. MARKRAM[2];
[2]Blue Brain Project, [1]EPFL, Lausanne, Switzerland

Abstract: The ventral posterolateral (VPL) nucleus of the thalamus serves as a relay center for the somatosensory system, receiving various forms of tactile and proprioception information from the periphery. These sensory signals are known to be mapped onto the somatosensory cortical surface in a topographic point-for-point correspondence. The VPL, a subcortical input to the somatosensory cortex, has also been observed to exhibit a similar somatotopy, and thus, we hypothesized a corresponding distribution of the physiology, morphology, and connectivity of the neurons. Here, we systematically investigated the electrophysiological and morphological characteristics of the VPL in rats of age postnatal 14-18 days. We obtained whole cell multi-patch clamp recordings of VPL relay cells in acute slices and stained and morphologically reconstructed the neurons. In addition, we used retrograde tracing to map the parallel connectivity between the VPL relay cells and associated primary cortical regions. The VPL relay cell data is being integrated into the *in silico* model of the rat somatosensory cortex (Markram et al. 2015) to reconstruct and simulate this thalamocortical pathway. We explored how the heterogeneity of functional and morphological properties of the VPL and their connectivity properties may impact sensory perception.

Disclosures: **J. Yi:** A. Employment/Salary (full or part-time): EPFL. **E. Iavarone:** A. Employment/Salary (full or part-time): EPFL. Other; The ETH Board Funding to the Blue Brain Project, European Union Seventh Framework Program (FP7/2007-2013) under grant agreement no. 604102 (HBP), The EPFL Blue Brain Project. **C. O'Reilly:** A. Employment/Salary (full or part-time): EPFL. Other; The EPFL Blue Brain Project, The ETH Board Funding to the Blue Brain Project, European Union Seventh Framework Program (FP7/2007-2013) under grant agreement no. 604102 (HBP). **R. Perin:** A. Employment/Salary (full or part-time): EPFL. **H. Markram:** A. Employment/Salary (full or part-time): EPFL. Other; The EPFL Blue Brain Project, The ETH Board Funding to the Blue Brain Project, European Union Seventh Framework Program (FP7/2007-2013) under grant agreement no. 604102 (HBP).

Poster

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Title: A data-driven computational model of relay cells of the ventral posterolateral nucleus of the thalamus

Authors: *E. IAVARONE[1,2], C. O'REILLY[1,2], J. YI[2,3], H. MARKRAM[1,2,3], S. HILL[1,2]; [1]Blue Brain Project, Geneva, Switzerland; [2]École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; [3]Brain Mind Inst., Lausanne, Switzerland

Abstract: Thalamic sensory nuclei are important relay stations along the lemniscal and paralemniscal pathways, which transfer somatosensory information from the periphery to the neocortex. However, the sensory thalamus is also part of the thalamocortical loop, a circuit that sustains sleep rhythms, wakefulness, alertness, and consciousness, in cooperation with the thalamic reticular nucleus and brainstem modulatory nuclei. Our long-term goal is to investigate these brain functions in a detailed computational model of the thalamocortical loop. We first focused on thalamocortical (TC) cells of the ventral posterolateral nucleus (VPL), the direct counterpart of the neocortical microcircuit model of the hindlimb somatosensory cortex proposed by Markram *et al*. (2015).
We used the NEURON simulator to build data-driven biophysical models of TC cells. Model construction and validation were achieved using open-source software and well-established pipelines, in reproducible steps organized in Jupyter (IPython) notebooks. Features characterizing the two main firing regimes of TC cells (*i.e*. tonic firing and bursting) were extracted from electrophysiological recordings. The free parameters describing ion channels densities and distributions were constrained using a multi-objective optimization strategy already applied by our group (Van Geit *et al*., 2016). We validated our model by observing that values describing cell properties, not fitted during the model construction phase, fell within expected physiological ranges. The final set of parameters was applied in combination with different 3D

morphologies and the resulting models were considered acceptable if the firing responses fell in the range of experimental variability.
Our methodological approach makes the process of building data-driven *in silico* neurons reproducible and easily adaptable. The results of the simulations replicated many experimental findings, including the firing behavior reported in the literature and observed in our multi-patch recordings.
Markram, Henry, et al. "Reconstruction and simulation of neocortical microcircuitry." Cell 163.2 (2015): 456-492.
Van Geit, Werner, et al. "BluePyOpt: Leveraging open source software and cloud infrastructure to optimise model parameters in neuroscience." arXiv preprint arXiv:1603.00500 (2016).

Disclosures: **E. Iavarone:** None. **C. O'Reilly:** None. **J. Yi:** None. **H. Markram:** None. **S. HIll:** None.

Poster

526. Somatosensory Cortical Circuits: Blue Brain Project

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Title: A data-driven unifying model of synaptic variability and multi-vesicular in neocortical microcircuitry

Authors: ***J. RAHMON**, G. CHINDEMI, R. PERIN, M. REIMANN, M. NOLTE, H. MARKRAM, E. MULLER, S. RAMASWAMY;
EPFL Blue Brain Project, Geneve, Switzerland

Abstract: Synaptic connections between neurons are characterized by a diversity of efficacy and reliability. Here, we focused on connections between layer 5 thick-tufted pyramidal cells (L5TTPCs), where the synaptic anatomy and physiology are well characterized experimentally.

We used an extension of the classical quantal model of synaptic transmission to examine how synaptic variability and muti-vesicular release (MVR) contribute to the efficacy and reliability of L5TTPC connections. We implemented a model of synaptic variability and MVR into morphologically-detailed *in silico* reconstructions of L5TTPC connections, and systematically calibrated the synaptic parameters by iteratively comparing the behavior of *in silico* connections against *in vitro* biological data. Next, we applied a novel technique to predict additional features of synaptic variability, such as intra-synapse correlations between the size of the readily-releasable pool and the postsynaptic response to the release of a single vesicle, for which biological data is either sparse or lacking. Finally, we implemented the validated model of synaptic variability and MVR into a digital reconstruction of neocortical microcircuitry. This work provides detailed insights into the impact of synaptic variability and MVR on properties of neocortical network activity, including spiking probability, and spike timing reliability of neurons.

Disclosures: **J. Rahmon:** None. **G. Chindemi:** None. **R. Perin:** None. **M. Reimann:** None. **M. Nolte:** None. **H. Markram:** None. **E. Muller:** None. **S. Ramaswamy:** None.

Poster

526. Somatosensory Cortical Circuits: Blue Brain Project

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Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano

Title: The human brain project brain simulation platform

Authors: ***F. SCHUERMANN**[1], J.-D. COURCOL[1], J. HELLGREN KOTALESKI[2], H. MARKRAM[1];
[1]Campus Biotech, EPFL - Blue Brain Project, Geneva, Switzerland; [2]Dept. Computat. Sci. and Technol., KTH Royal Inst. of Technol., Stockholm, Sweden

Abstract: We present an overview of the core functionalities of the Human Brain Project (HBP) Brain Simulation Platform (BSP), on behalf of all contributors. The BSP was developed as one of six internet-accessible ICT platforms for collaborative brain research being developed by the European HBP, with the goal of providing scientists with new user-friendly tools to reconstruct and simulate scaffold models of various neuron types and whole brain tissue in a data-driven fashion. Its development is done in a tight co-design loop between science and engineering within the HBP consortium, to stimulate the required substantial technical and scientific innovations. One important co-design driver has been the reconstruction and simulation of the neocortical microcircuitry of the Blue Brain Project (Markram et al., Cell, 2015), and the recent application of these techniques and workflows to other brain regions, notably the cerebellum, hippocampus and basal ganglia. The BSP was released to the public in April, 2016, (https://www.humanbrainproject.eu/pl/sp6-brain-simulation-platform) providing intricate pipelines for reconstruction and simulation packaged into web-accessible workflows, or showcased as use cases. Many of the fundamental software packages that the BSP relies on and develops are freely available as open source. Using these tools and workflows, the BSP hosts team-science Collabs for collaborative building of scaffold models of different neurons, synapses and brain regions. The unique functionality of the BSP accelerates collaborative brain research worldwide and allows novel questions to be addressed, which were previously inaccessible.
H. Markram et al. (2015). Reconstruction and Simulation of Neocortical Microcircuitry. Cell 163:2, 456 - 492. doi: 10.1016/j.cell.2015.09.029

Disclosures: F. Schuermann: None. **J. Courcol:** None. **J. Hellgren Kotaleski:** None. **H. Markram:** None.

Poster

526. Somatosensory Cortical Circuits: Blue Brain Project

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 526.12/TT3

Topic: D.03. Somatosensation: Touch

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Advanced Grant of the European Research Council GUDHI

SNF NCCR "Synapsy"

Title: Quantitative topological analysis of neuronal trees

Authors: *L. KANARI[1], P. DLOTKO[2], M. SCOLAMIERO[3], R. LEVI[4], J. SHILLCOCK[1], K. HESS[3], H. MARKRAM[1];
[1]Blue Brain Project, EPFL, Geneve, Switzerland; [2]DataShape, INRIA Saclay, Ile-de-France, Paris, France; [3]Lab. for Topology and Neurosci. at the Brain Mind Institute, EPFL, Lausanne, Switzerland; [4]Inst. of Mathematics, Univ. of Aberdeen, Aberdeen, United Kingdom

Abstract: Simulations of the mammalian neocortex, such as those performed by the Blue Brain Project, rely fundamentally on accurate neuronal morphologies. The shape of neuronal arborizations is a key feature for the classification of neurons, and defines amongst other aspects their physical connectivity and functionality. Yet an efficient method for quantitatively analyzing the spatial structure of such trees has been difficult to establish.
The wide diversity of neuronal morphologies in the brain, even for cells identified by experts as of the same type, renders an objective classification scheme a challenging task. We propose a new topological method for quantitatively capturing the branching shapes of neurons, the Topological Morphology Descriptor (TMD), that overcomes the limitations of existing techniques such as feature extraction. The TMD of neuronal trees allows different neuronal populations to be clearly distinguished, including those whose visual appearance is only subtly different, and therefore provides an objective classification of cells into discrete morphological classes.
This method is applicable to any tree-like structure, and we demonstrate this by applying it to groups of mathematical random trees as well as neuronal trees. Our results show that the TMD of tree shapes is highly effective for reliably and efficiently distinguishing different groups of trees. We also propose an extension to the method that can be used to track the morphological evolution of trees.

Disclosures: **L. Kanari:** None. **P. Dlotko:** None. **M. Scolamiero:** None. **R. Levi:** None. **J. Shillcock:** None. **K. Hess:** None. **H. Markram:** None.

Poster

526. Somatosensory Cortical Circuits: Blue Brain Project

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Title: Data-driven reconstruction of mesoscale connectivity in a whole mouse-brain model

Authors: *M.-O. GEWALTIG, C. EROE, D. KELLER, H. MARKRAM;
Blue Brain Project, Ecole Polytechnique Federale De Lausanne, Geneve, Switzerland

Abstract: Retrograde Recombinant Adeno-Associated Virus (rAAV) is a virus used to trace neural pathways by expressing fluorescent proteins in the cell bodies, dendrites, and axons of injected neurons. Recently, the Allen Institute for Brain Science (Oh et al. 2014) published an extensive dataset of multiple rAAV injections along with the estimated connection probabilities between 213 areas from the Allen Brain Atlas (ABA) and their statistical significance in terms of P-values.In this contribution, we present an alternative analysis of the same data with the aim to estimate the long-range connectivity of a point neuron network of whole mouse brain (Eroe et al. 2015). Our workflow consisted of two steps: In the first step, we generated an approximate model of the positions of all excitatory and inhibitory neurons in the mouse brain, based on the Allen Brain Atlas. In the second step, we used the raw rAAV voxel data to create synaptic connections between pairs of neurons using an acceptance-rejection method. To compare our mesoscale connectivity model with the previously published connectome from the ABI, we determined the connection probabilities between the same 213 brain regions as analyzed by Oh et al. Assuming that the strength of an inter-areal connection proportional to the number of mutual synaptic contacts, we counted the number of mutual synaptic contacts for each pair of brain regions. Finally, we compared our synapse-based connectome to the ABI mesoscale connectome, focusing on the strongest 50% of the connections. This allowed us to to identify connections that are common in both connectomes, connections that are exclusively predicted by Oh et al. and connections that are exclusively predicted by our approach. Both connectomes showed similar patterns of connectivity, which was expected as both used the same rAAV injection datasets. We also observed a similar near log-normal distribution of the connections strengths (in number of synapses). Current work focusses on integrating other data-sets (Markram et al. 2015) to also obtain microscale connectivity between cortical neurons.
References:
Markram, H. et al. 2015. Cell 163 (2): 456-92. doi:10.1016/j.cell.2015.09.029.
Oh et al. 2014. Nature 508 (7495): 207-14. doi:10.1038/nature13186.
Eroe et al. 2015. Society for Neuroscience Abstracts 515.06, Chicago, IL

Disclosures: **M. Gewaltig:** None. **C. Eroe:** None. **D. Keller:** None. **H. Markram:** None.

Poster

526. Somatosensory Cortical Circuits: Blue Brain Project

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Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano

Title: Network state-dependence of synaptic transmission in neocortical microcircuitry

Authors: *S. RAMASWAMY, J. G. KING, M. W. REIMANN, H. MARKRAM, E. MULLER; EPFL - Blue Brain Project, Geneva, Switzerland

Abstract: The neocortex contains a rich diversity of excitatory and inhibitory neurons. Decades of studies have enriched our understanding of the principles of neuronal and synaptic organization in neocortical networks. However, detailed knowledge on how network activity states regulate neuronal physiology and synaptic transmission is still lacking. The Blue Brain Project has developed a process to pragmatically integrate neuronal and synaptic organizing principles to digitally reconstruct the detailed anatomy and physiology of neocortical microcircuitry from sparse biological data. The *in silico* reconstruction of neocortical microcircuitry comprises a volume of 0.29 mm^3, ~31,000 neurons distributed across 6 layers, 55 layer-specific morphological and 207 morpho-electrical neuron subtypes, and ~8 million synaptic connections with ~37 million synapses. Simulations of spontaneous activity in the reconstruction revealed a spectrum of network states spanning synchronous neuronal activity on one end, and asynchronous activity on the other. The *in silico* reconstruction predicts the physiology of 31,000 neurons, and their synaptic connections in a spectrum of network activity states mediated by tonic depolarization and extracellular Ca^{2+} ranging from *in vitro* (~2 mM) to *in vivo* (~1.2 mM) levels. Experimentally testable predictions provide further insights into network state-dependence of the efficacy and short-term dynamics of excitatory and inhibitory synaptic transmission in neocortical microcircuitry across a range of neuronal depolarization and extracellular Ca^{2+} levels. We propose the reconstruction as a powerful complementary approach to existing experimental techniques to foster a systematic characterization of the structure and function of neocortical microcircuitry.

Disclosures: **S. Ramaswamy:** None. **J.G. King:** None. **M.W. Reimann:** None. **H. Markram:** None. **E. Muller:** None.

Poster

526. Somatosensory Cortical Circuits: Blue Brain Project

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Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano

Title: In silico voltage sensitive dye imaging in a digital model of somatosensory cortex.

Authors: ***T. H. NEWTON**[1], M. ABDELLAH[2], E. MULLER[2], F. SCHUERMANN[2], H. MARKRAM[2];
[1]EPFL BBP, Geneva, Switzerland; [2]BBP, EPFL, Lausanne, Switzerland

Abstract: We report on an in silico implementation of voltage sensitive dye imaging (VSDI) for exploring and validating mesoscopic neural activity in a biologically detailed digital reconstruction of rat somatosensory cortex. The cortical model comprised a network of ~775,000 neurons arranged in a five by five hexagonal grid of connected functional microcircuit units, spanning a patch of simulated tissue 1990 μm x 1840 μm in area and 2000 μm deep. Two methods of acquiring in silico fluorescence signals were compared for computational performance, namely Monte Carlo simulation and a Beer-Lambert law-based approximation of light scattering and absorption in tissue. We evaluated the behavior of our in silico VSDI model against several findings reported in the literature, including sublinear summation of paired stimulation, activity spread velocity, and state-dependent stimulus response. Furthermore, leveraging the flexibility of our digital model, we addressed two unresolved questions concerning the origins of the VSD signal: 1) the relative importance of spiking activity vs. subthreshold fluctuations in membrane potential, and 2) the respective contributions of excitatory and inhibitory cells.

Disclosures: **T.H. Newton:** None. **M. Abdellah:** None. **E. Muller:** None. **F. Schuermann:** None. **H. Markram:** None.

Poster

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Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano

Title: Automated point-neuron simplification of data-driven microcircuit models

Authors: ***C. RÖSSERT**[1], C. POZZORINI[2], G. CHINDEMI[1], A. P. DAVISON[3], C. EROE[1], J. KING[1], T. H. NEWTON[1], M. NOLTE[1], S. RAMASWAMY[1], M. W. REIMANN[1], M.-O. GEWALTIG[1], W. GERSTNER[2], H. MARKRAM[1], I. SEGEV[4], E. MULLER[1];
[1]Blue Brain Project, EPFL, Geneve, Switzerland; [2]Lab. of Computat. Neurosci., EPFL, Lausanne, Switzerland; [3]Unité de Neurosciences, Information et Complexité, Ctr. Natl. de la Recherche Scientifique, Gif sur Yvette, France; [4]Dept. of Neurobio. and Admond and Lily Safra Ctr. for Brain Sci., The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: A first detailed draft of digitally reconstructed and numerically simulated interactions in ~1/3 of a cubic millimeter of the rat neocortex has recently been reported (Markram et al. 2015). This work integrated the current state of experimental knowledge about the detailed 3D anatomy and physiology of the various neuron types and their synaptic properties and connectivity. On the other hand, for large-scale network simulations, point-neuron models are typically used for describing and analyzing network dynamics and functions. The properties and connectivity structure of point neuron models, for the most part, have not been constrained by biological data and thus were using ad hoc assumptions. This makes some of the mathematically tractable models somewhat disconnected from experimental neuroscience. To bridge the gap between these two extremes (the detailed and the oversimplified), we aimed to derive point-neuron network models from data-driven detailed network models in an automated, repeatable

and quantitatively verifiable manner. The simplification occurs in a modular workflow, in an *in vivo*-like state. First, synapses are moved from dendrites to the soma while correcting for dendritic filtering using low-pass filters for the synaptic current with parameters computed numerically in a morphology-type specific manner. Next, point-neuron models for each neuron in the microcircuit are fitted to their respective morphologically detailed counterparts. Here, generalized integrate-and-fire point neuron models are used, leveraging on a recently published fitting toolbox (Pozzorini et al. 2015). The fits are constrained by currents and voltages computed in the morphologically detailed reference neurons with soma-located synapses. Benchmarking the simplified network model to the detailed microcircuit model for a range of simulated *in vivo* and *in vitro* protocols, we found good agreement for both quantitative and qualitative aspects. Our approach not only makes it possible to continuously update the simplified circuit as the detailed network integrates new data but the modularity of the simplification process also makes it applicable to other point-neuron and synapse models and network simulators. In addition to providing strong validation for carefully-reduced point neuron network models, our approach is fundamentally important and informative, in particular in cases when network functionalities are lost during the simplification pipeline. Missing functionalities can illustrate the contributions of specific synaptic and cellular (anatomical and physiological) properties to the overall computations implemented by the network.

Disclosures: **C. Rössert:** None. **C. Pozzorini:** None. **G. Chindemi:** None. **A.P. Davison:** None. **C. Eroe:** None. **J. King:** None. **T.H. Newton:** None. **M. Nolte:** None. **S. Ramaswamy:** None. **M.W. Reimann:** None. **M. Gewaltig:** None. **W. Gerstner:** None. **H. Markram:** None. **I. Segev:** None. **E. Muller:** None.

Poster

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Title: The connectome is also a mathematical problem

Authors: ***M. W. REIMANN**, H. MARKRAM;
Blue Brain Project, Geneve, Switzerland

Abstract: Obtaining the synaptic connectivity between neurons is one of the biggest challenges of modern neuroscience. Various approaches involving a considerable amount of effort and resources have been devoted to experimentally map the connectivity between neurons. Yet, each of these approaches only sample a minuscule and potentially biased fraction of any given brain region, and conversely, a map of connectivity between neurons large enough to understand brain function still cannot be fully established. Here, we argue that full experimental mapping of all connections may not be necessary since known principles of biological organization already constrain which connections can and cannot exist. To know the state of a system, exhaustive sampling is only required if it is completely random, however, the brain like any other biological system is characterized by structure. We show mathematically, how the application of known rules of biological structure and simple physical limitations - such as limited axon lengths and bouton densities, multi-synaptic connections and the requirement of axo-dendritic overlap - constrain the neocortical connectome. We measure the remaining entropy of the connectome before and after the application of constraints and further show which strategic measurements are expected to most reduce it in the future.

Disclosures: **M.W. Reimann:** None. **H. Markram:** None.

Poster

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Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano

Title: Multi-level brain region reconstruction and simulation on supercomputers: Enhancements for performance, scalability, and usability.

Authors: ***J. G. KING**[1], P. KUMBHAR[1], M. HINES[2], A. DEVRESSE[1], T. SCHUMANN[1], A. OVCHARENKO[1], S. YATES[1], F. DELALONDRE[1], F. SCHUERMAN[1], H. MARKRAM[1]; [1]Blue Brain Project, Brain Mind Institute, EPFL, 1202 Geneva, Switzerland; [2]Dept. of Neurobio., Yale Univ., New Haven, CT

Abstract: The Blue Brain Project has developed a data driven process to digitally reconstruct and simulate a piece of neocortical tissue, which faithfully reproduces an array of laboratory experiments. Efforts continue to extend the capabilities of tools to support greater variety and biological accuracy of these reconstructions. The first part of the workflow consists of implementing applications for large-scale neuronal network reconstructions including millions of neurons. New additions to the building capabilities have first been focused on increasing the level of biological details and thus the faithfulness of model. As such support for more complex rotations implemented as quaternions has been added to better capture the anatomy of curved tissue rather than flat tissue slices prior. This was done with an initial use case involving regions of the Hippocampus. Parallel to these scientific developments the usability of the building pipeline has been increased by adding the capability to output model deltas at each stage of the building pipeline and make all microcircuit reconstruction features accessible through the Human Brain Project "Software as a Service (SaaS)" Collaboratory for ease of use by the community. To leverage the recent improvements of the model-building pipeline, new simulation capabilities have been added to brain simulators at three different scales. At the coarsest scale, a parallel implementation of the NEST import module has been implemented and tested on up to 28 racks of the Juqueen supercomputer to allow the faithful model building of the mouse brain size with both short and long range biological data connections. At the intermediate scale, CoreNeuron has been open sourced on github to support development of more efficient and scalable simulation of large neuronal networks (up to 550 million neurons) on 48 racks of the MIRA supercomputer. Finally, the STEPS simulator has been extended to support the distributed execution of coupled stochastic reaction-diffusion and eField model on up to 1,000 cores.

Disclosures: **J.G. King:** None. **P. Kumbhar:** None. **M. Hines:** None. **A. Devresse:** None. **T. Schumann:** None. **A. Ovcharenko:** None. **S. Yates:** None. **F. Delalondre:** None. **F. Schuerman:** None. **H. Markram:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 527.01/TT10

Topic: D.04. Olfaction and Taste

Title: Neural mechanisms underlying odor preference choice in *Drosophila* larva

Authors: *Y. DAIRYO, K. EMOTO;
The Univ. of Tokyo, Tokyo, Japan

Abstract: The sensory-evoked behavior of animals is flexible and can be changed dramatically during development, yet the underlying mechanisms remain obscure. We focused on the odor preference behaviors in *Drosophila* larvae as a model system and established a quantitative assay system for the larval odor preference behavior. Using this assay system, we found that the third instar larvae dramatically change their odor preference during the late larval stages. For example, foraging larvae (72-96 hours after egg laying) are strongly attracted to propionic acid, whereas wandering larvae (96-120 hours after egg laying) show less interest to the same odor. This suggests that neural circuits that determine the odor preference might be modified during 24 hours prior to metamorphosis. To elucidate neural circuits underlying the preference change, we attempted to identify neurons involved in the odor preference choice by genetically manipulating neuronal functions. We recently identified two pairs of candidate neurons involved in the preference change. The wandering larvae failed to change the odor preference if the candidate neurons were functionally silenced. Moreover, the candidate neurons projected to the mushroom body neurons, the neurons involved in various innate behaviors and associative olfactory learning in *Drosophila*, suggesting that the MB neurons serve as a modification site for the odor preference change. We would like to discuss how the brain makes decision and also changes the decision against the same sensory information.

Disclosures: **Y. Dairyo:** None. **K. Emoto:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: KAKENHI Grant 23680044

KAKENHI Grant 25115732

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Title: Interaction between cell-type specific excitation and inhibition in the *Drosophila* mushroom body circuit

Authors: *K. INADA[1,2], H. KAZAMA[1,2];
[1]RIKEN BSI, Wako Saitama, Japan; [2]Grad. school of arts and sciences, Univ. of Tokyo, Bunkyo-ku, Japan

Abstract: Information about external stimuli is encoded as spatiotemporal patterns of neural activity in the periphery. These neural representations are transformed progressively in multiple brain regions. Different types of neurons in a region play different roles in computation, but the underlying biophysical mechanisms remain unclear. To understand the mechanism, it is essential to place individual neurons in the context of a circuit and examine how synaptic transmissions interact in these cells. In practice, this involves manipulating specific types of inputs to a target cell, which can be difficult to achieve.

Here we use the mushroom body (MB) circuit of *Drosophila melanogaster*, which is suitable to achieve this goal. The MB encodes odors sparsely and plays an essential role in olfactory association. The principal neurons of the MB, the Kenyon cells (KCs), are morphologically divided into three subtypes: α/β, α'/β' and γ. They receive excitatory input from a subset of projection neurons (PNs) in the antennal lobe and inhibitory input from a giant GABAergic anterior paired lateral (APL) neuron in the MB.

We started by examining how excitatory inputs are integrated in KCs. We expressed an optogenetic probe in PNs to manipulate their activity while performing whole-cell patch-clamp recording from postsynaptic KCs. Because each PN sends dendrites exclusively to a single glomerulus in the antennal lobe, the activity of a specific type of PNs can be manipulated by targeting a specific glomerulus with light. Glomeruli were stimulated either with a pulsed laser or wide-field blue light. We found three major rules of signal processing. First, synaptic transmission between PNs and KCs is faithful: KCs respond in proportion to the PN firing rate. Second, KCs integrate inputs from multiple PNs linearly. Third, responses of KCs are largely determined by directly-connected PNs with little influence from indirectly-connected (lateral) PNs.

As we increased the strength of PN input to the MB, APL neuron started to provide feed-back inhibition to KCs. We found that although APL neuron morphologically innervates the entire MB, it can inhibit KCs locally: only a subset of KCs was inhibited depending on the level of PN activity. Notably, this inhibition was recruited mainly by α'/β' KCs. Because optogenetic stimulation of APL neuron inhibited all KC subtypes similarly, our data together suggest that input from KCs to APL neuron is the origin of specificity.

Our results reveal cell-type specific circuit mechanisms that shape the activity of KC subtypes, each contributing to different aspects of olfactory memory.

Disclosures: K. Inada: None. **H. Kazama:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: DFG SPP 1392 "Integrative Analysis of Olfaction"

Title: Postmetamorphic plasticity of the mushroom bodies

Authors: *B. TREBELS[1], S. DIPPEL[2], E. A. WIMMER[2], J. SCHACHTNER[1];
[1]Deptartment for Biology, Animal Physiol., Philipps-University Marburg, Marburg, Germany;
[2]Dept. of Developmental Biol., Georg-August-University Göttingen, Göttingen, Germany

Abstract: With its fully sequenced genome and the susceptibility for reverse genetics based upon RNA interference (RNAi), *Tribolium castaneum* is best suited to study the development and plasticity of the nervous system. While plasticity can be provided by various mechanisms, we focus on ongoing cell proliferation in the adult brain. It is well established that neurogenesis persists in the mushroom bodies (MB) of adult insects, including beetle *T. castaneum* where neuroblasts giving birth to MB Kenyon-cells remain active for more than one month after adult eclosion. To label cell proliferation in the adult beetle we successfully adapted the 5-ethyl-2'-deoxyuridine (EdU) technique to living beetles. Combined with immunohistochemistry against the glia-cell marker reversed-polarity and the use of transgenetic lines expressing neuron- and/or glia-specific markers, we labeled the progenies of adult persisting neuroblasts, determined their identity and counted the newborn Kenyon cells in within the first week after adult eclosion to determine the proliferation rate.
In several studies it was proposed that newborn neurons of MBs may play a role during olfactory processing and learning. We combined the EdU-staining with olfactory stimulation using the leaf alcohol cis-3-hexen-1-ol and again determined the proliferation rate. Our data suggest at least two phases. Direct after adult eclosion, proliferation is independent from stimulation with the leaf alcohol, while after about three days, proliferation is influenced by olfactory stimulation.
To further investigate MB plasticity, we plan to use other odors, including the beetle's aggregation pheromone 4,8-dimeythydecanal (DMD), odor deprivation and knockdown of the general olfactory receptor (Orco) via systemic RNAi.

Disclosures: **B. Trebels:** None. **S. Dippel:** None. **E.A. Wimmer:** None. **J. Schachtner:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

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European Molecular Biology Organization (EMBO)

Swiss National Science Foundation (SNF)

Title: Illuminating the function of inhibitory microcircuits in the zebrafish homolog of olfactory cortex

Authors: ***T. FRANK**, R. W. FRIEDRICH;
Friedrich Miescher Inst., Basel, Switzerland

Abstract: The brain creates dynamic representations of the sensory environment by extracting stimulus features at early processing stages and synthesizing more abstract object representations in higher brain areas. We dissect the function of neuronal microcircuits in higher olfactory brain areas in order to identify elementary computations of basic cortical circuits and to analyze the underlying cellular mechanisms. To this end, we use a combination of genetic, electrophysiological and optical approaches to visualize, record, and manipulate different types of interneurons (INs) in different subdivisions of the posterior zone of the dorsal telencephalon (Dp) of adult zebrafish. This brain area is homologous to olfactory cortex in mammals and assumed to be involved in olfactory object representations and associative memory. We identified two types of INs with similar electrophysiological properties that are differently connected to other neurons in Dp and also impact excitatory responses differently. These results shed light on cell-type specific computations in a higher-order olfactory area and provide insights into canonical computations performed by basic cortical circuits.

Disclosures: **T. Frank:** None. **R.W. Friedrich:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: K-INBRE (P20GM103418)

Title: Odor and stress-induced pERK expression reveal distinct neural activity patterns in the forebrain of adult zebrafish

Authors: *B. A. PORTER, G. STEVENS, T. MUELLER;
Div. of Biol., Kansas State Univ., Manhattan, KS

Abstract: Altered smell sensation is a hallmark of human affective disorders. Schizophrenia and bipolar disorder, for example, are characterized by deficits in odor discrimination. Likewise, increased sensitivity to smell accompanies fear and anxiety disorders. Despite their medical relevance, the neural circuits mediating olfaction and emotion in higher forebrain regions such as the telencephalon, hypothalamus, and habenula, are barely understood. Zebrafish, due to its small size and amenability to genetic manipulations, has become an important model to dissect neural circuits of sensory processing and behavior. To this date, however, the functional organization of the olfactory pallial (cortical) nuclei in zebrafish and their relationships to the mammalian situation remains unresolved. To shed light on the functional organization of these and other odor-processing nuclei in the zebrafish forebrain, we began to investigate neural activity patterns with an antibody against **p**hosphorylated **e**xtracellular signal-**r**egulated **k**inase (pERK) as a postmortem readout of neural activity in odor-driven experimental paradigms. Specifically, we compare pERK expression patterns after stress induced by netting with those resulting from the application of qualitative different odors. When compared with each other, the three different paradigms (stress, food extract, and alarm substance) reveal distinct expression patterns supporting an overall conserved functional forebrain organization. In this future, we will use this method to further functionally characterize the zebrafish forebrain in regard to odor-processing nuclei. The results of this study will provide a solid foundation to dissect neural circuits of odor-induced emotion and socio-sexual (reproductive) behavior in this important model organism.

Disclosures: B.A. Porter: None. **G. Stevens:** None. **T. Mueller:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: MH094360

Title: Connectome of mouse olfactory system

Authors: ***M. ZHU**, B. ZINGG, L. GOU, M. BIENKOWSKI, H. HINTIRYAN, H. DONG; USC, Los Angeles, CA

Abstract: Olfaction is important for survival and quality of life in both animals and humans. How odor information is encoded in the olfactory bulb (OB) has been extensively studied in the last three decades. Yet, it remains largely unclear how olfactory information is processed sequentially in other brain structures within the olfactory network that includes the anterior olfactory nucleus (AON), piriform cortex (PIR), cortical amygdalar areas (COA), and olfactory tubercle (OT). Here, we report our effort to construct the mouse olfactory connectome. Coinjections of anterograde and retrograde tracers were placed into all brain regions that receive direct input from the main olfactory bulb (MOB). Our preliminary data, together with those in the literature (Haberly and Price, 1978; Luskin and Price, 1983), suggest that all olfactory cortical areas are heavily interconnected and share direct connections with the lateral entorhinal cortex (ENTl, which is classically considered a part of the olfactory cortical area), the hippocampus (especially with the ventral and intermediate CA1 and subiculum), and the agranular insular cortex (AI). To systematically and accurately map and quantify these connectivity pathways, we developed informatics algorithms to register all microscopic images to a standard mouse brain atlas, to extract and quantify tracer signal, to perform network analysis, and to construct a connectivity matrix. This comprehensive olfactory connectome will facilitate hypotheses regarding the functional relevance of the entire olfactory system.

Disclosures: **M. Zhu:** None. **B. Zingg:** None. **L. Gou:** None. **M. Bienkowski:** None. **H. Hintiryan:** None. **H. Dong:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: D.04. Olfaction and Taste

Support: NIH/NIDCD Grant R01-DC014426

NIH/NIA Grant P30-AG013854

Title: Structure of the human central olfactory system

Authors: J. LI[1], A. WEIBMAN[1], T. BOZZA[4], E. BIGIO[2], M.-M. MESULAM[3], *J. A. GOTTFRIED[1], C. GEULA[3];
[1]Neurol., [2]Pathology, [3]Cognitive Neurol. & Alzheimer Dis. Ctr., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [4]Neurobio., Northwestern Univ. Weinberg Col. of Arts and Sci., Evanston, IL

Abstract: There are profound interspecies variation in olfaction, with corresponding differences in the central olfactory apparatus. While numerous components of the central olfactory system are preserved when rodent species are compared to the primate, the layout of these components differs greatly. Most of our understanding of the anatomy of the central olfactory system in the primate is derived from the macaque brain. The purpose of this study was to investigate the detailed structure of this system in the human brain. Full hemispheric sections from three normal brains were processed to visualize cell bodies using the Cresyl violet Nissl stain. One brain was embedded in celloidin, and alternate thick sections were stained for Nissl, or for myelin using the Loyez method. Throughout most of its course, the olfactory tract (OTr) displays a triangular shape in cross section, with myelinated axons concentrated in the periphery. Once the tract reaches the most posterior aspects of the olfactory sulcus in the medial orbitofrontal cortex (OFC), the apex of the triangle elongates with two streams of axons directed dorsally toward OFC. More posteriorly, the bases of the triangle merge into a primary bundle of axons directed laterally, and a minor bundle directed medially, the latter contributing axons to medially streaming white matter. The lateral bundle further differentiates into two streams, with a minor component directed laterally toward the insula, and a major component directed ventrally toward the temporal pole (TP), where it enters layer I of cortex. Another distinct target of the OTr is the anterior olfactory nucleus (AON), appearing first as a small collection of neurons within the posterior aspects of OTr, just before OTr enters cortex. More posteriorly, the AON is located at the base of the olfactory sulcus as several relatively large collections of neurons surrounded by OTr axons. Just dorsal and posterior to AON lies the OFC component of piriform olfactory cortex (PCx). As in the macaque, the PCx trifurcates from a “root” near the limen insula (i.e., the junction where the orbitofrontal, temporal and insular cortices first join), contributing

components to the OFC, the insula (Ins), and the TP. These three components occupy a large region, and the PCx protrudes in small but distinct gyri in the three cortical areas. In summary, the components of the central olfactory system described in the primate can be identified in the human brain. However, the extent of the area occupied by these components in the human is considerably expanded, underscoring the widespread anatomical diversity of afferent projections from the OTr that define the human olfactory system.

Disclosures: J. Li: None. **A. Weibman:** None. **T. Bozza:** None. **E. Bigio:** None. **M. Mesulam:** None. **J.A. Gottfried:** None. **C. Geula:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: NHMRC Project Grant 1009382

NHMRC Project Grant 1050832

Title: Odor processing by a feedforward inhibitory circuit in the piriform cortex

Authors: N. SUZUKI, M. L. S. TANTIRIGAMA, H. H.-Y. HUANG, *J. M. BEKKERS; John Curtin Sch. Med. Res., Acton, Australia

Abstract: The piriform cortex (PC) is a trilaminar paleocortex that is important for constructing and remembering odor percepts. We use the PC as an anatomically simple model system for studying canonical cortical circuits that have persisted through evolution. In this project we focused on feedforward inhibitory synaptic circuits which, in the PC, are largely confined to the upper part of layer 1 (layer 1a), where they are readily accessible ‘in vivo’ using two-photon-targeted patch clamping and calcium imaging. We describe the properties of a prominent feedforward circuit in layer 1a of the anterior PC, including the response of this circuit to odors. METHODS. The anterior PC of GAD67-GFP mice aged 35-75 days was surgically exposed under urethane or fentanyl anesthesia, in accordance with institutional guidelines. Targeted whole-cell recordings were obtained from identified layer 1a neurogliaform (NG) and horizontal (HZ) cells using a two-photon microscope. Some experiments used ‘in vivo’ two-photon calcium imaging with the indicator Cal-590 AM. An olfactometer was used to apply up to 15 monomolecular odorants. Some experiments were done using acute brain slices (300 microns thick). RESULTS. NG and HZ cells were identified ‘in vivo’ by their soma location, GFP

fluorescence intensity and intrinsic electrical properties. Both NG cells and HZ cells were broadly excited by odors and exhibited oscillations in subthreshold membrane potential that were phase-locked to respiration. However, they differed strongly in the dynamics of their odor response: NG cells fired early after odor onset (<0.5 s), whereas HZ cells fired late (>1 s). This finding was also confirmed using calcium imaging. Whole-cell recordings 'in vivo' and in slices indicated that the delayed firing of HZ cells could be explained by their early inhibition by NG cells. By recording from layer 2 principal cells, we also found that NG and HZ cells provide phasic inhibition with characteristic dynamics. CONCLUSIONS. We have identified a cascading feedforward inhibitory circuit in the PC that likely refines the afferent excitatory input received by the PC from the olfactory bulb. Future work will need to explore the importance of this circuit for odor-driven behaviors.

Disclosures: N. Suzuki: None. **M.L.S. Tantirigama:** None. **H.H. Huang:** None. **J.M. Bekkers:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: NHMRC Project Grant 1050832

Title: Odor representation changes with repeated odor exposure in the mouse piriform cortex *In vivo*

Authors: *M. L. TANTIRIGAMA, J. M. BEKKERS;
The Australian Natl. Univ., Canberra, Australia

Abstract: Olfaction employs habituation to de-emphasize static or repetitive odor inputs in order to process novel, potentially more important odors. Previous work has shown that odors are represented in a sparse and odor-specific ensemble of neurons distributed in the piriform cortex (PC). However, it is unclear whether repeated exposure to a given odor can modulate odor representation, as a circuit manifestation of habituation, or whether the representation is static over time. Here, we study the neuronal responses to repetitive odor stimuli by simultaneously measuring the activity of up to 250 neurons in the PC of anesthetized mice using 2-photon calcium imaging, employing the calcium indicator dye Cal-520 AM. A given odorant excited a unique ensemble pattern of neurons (about 15 %) in layer 2, consistent with previous findings. Repeated application of the same odorants every three or six minutes produced a long-lasting

(>60 min) decline in the number of cells excited and in their response amplitude, indicative of habituation. This long-lasting habituated state was not apparent when a second odorant was presented and a different ensemble of neurons was excited. However, subsequent presentations of the second odorant produced a similar decline in activity as the first odorant, indicating that these changes are not generalized to the circuit activity but is odor-specific. We next tested whether habituation is inherited from the input received by the PC from the mitral/tufted (M/T) cells in the olfactory bulb (OB). Imaging activity in M/T cells revealed stable and reliable responses to repetitive odor stimuli, suggesting that habituation manifests from a local cortical mechanism. Previous work has implicated metabotropic glutamate receptor (mGluR) Group II/III involvement in habituation in the PC. Surprisingly, the long-lasting decline in neuronal responses was unchanged in the presence of the mGluR II/III antagonist (RS)-alpha-cyclopropyl-4-phosphonophenylglycine (CPPG), suggesting that this form of habituation is expressed via a different pathway and further pharmacological manipulations are necessary to identify its mechanism. The findings indicate long-lasting odor-specific changes to odor representation in the PC despite maintained input from the OB.

Disclosures: M.L. Tantirigama: None. **J.M. Bekkers:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

Location: Halls B-H

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Topic: D.04. Olfaction and Taste

Support: NIH Grant DC015139

Title: Somatostatin interneurons mediate activity gradients in piriform cortex

Authors: *A. M. LARGE, N. VOGLER, S. MIELO, A.-M. OSWALD;
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Abstract: Odor information is processed and encoded by the neural circuitry of the piriform cortex. Compared to primary sensory cortices, anterior piriform cortex (APC) lacks topographic representations of odor identity and appears to be a fairly homogenous structure in terms of connectivity and sensory processing. In order to observe the spatial structure of odor-evoked neuronal populations in APC, we utilized Targeted Recombination of Active Populations (TRAP) to fluorescently label neurons expressing the immediate early gene *c-fos* during odor presentation. We find that this active population decreases in density along the rostrocaudal (RC) axis of the APC. To investigate potential underlying mechanisms for an activity gradient, we

optically stimulated interneurons in APC slices from vGAT-ChR2 mice while recording IPSCs in piriform pyramidal cells, and demonstrate a clear caudal bias of inhibition onto pyramidal cells. Surprisingly, we also find that a majority of inhibitory interneurons receive biased inhibition but with an opposing RC gradient. Since FS cells are a major source of inhibition onto pyramidal cells, we believe that the activity gradient is due to modulation of inhibition onto FS cells. This suggests that the source of FS-cell inhibition needs to decrease along the RC axis. Using genetically targeted fluorescent labeling we find that somatostatin-expressing (SST) interneurons decrease in density along the RC axis. We also find that SST-cells can provide biased inhibition to inhibitory interneurons. Taken together, these results suggest a disinhibitory circuit mechanism supports an increasing gradient of inhibition resulting in enhanced odor-evoked activity of rostral pyramidal neurons but a decreased recruitment of caudal neurons.

Disclosures: **A.M. Large:** None. **N. Vogler:** None. **S. Mielo:** None. **A. Oswald:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: NIH grant DC015139

Title: Somatostatin interneurons in piriform cortex: expectations and limitations

Authors: ***A.-M. M. OSWALD**, A. M. LARGE, N. A. KUNZ, S. MIELO;
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Abstract: Inhibitory circuitry plays an important role in regulating cortical network activity during sensory processing. One major class of inhibitory interneuron expresses somatostatin (SST) and has been extensively characterized in neocortical sensory areas. Here we describe the anatomical distributions, intrinsic properties and inhibitory connectivity of SST-cells in piriform cortex. Our working hypothesis was that SST-circuitry in piriform cortex would be comparable to descriptions of SST-circuits in neocortical sensory areas. As expected, we found that SST-cells inhibit pyramidal cells, putatively identified parvalbumin (PV) interneurons as well as a number of unidentified interneuron classes. However, we also found a number of differences between classic neocortical descriptions of SST-cells and the properties and connectivity of SST-cells in piriform cortex. We compared SST-cells in two transgenic mouse lines, SST-IRES and GIN, with PV-expressing interneurons in G42 or PV-IRES mice. We found that regular-spiking

SST-cells in the GIN-line are sparse in piriform cortex. Rather, a majority of SST-IRES cells had electrophysiological properties similar to fast-spiking, PV interneurons. Furthermore, in contrast to reports from neocortex, we find that SST-cells can strongly inhibit each other. Finally, we find that SST-cells inhibit both distal dendrites and proximal somatic regions of pyramidal cells nearly equivalently. Altogether, these findings have important implications for both the application of current experimental methodologies and the interpretation of findings with respect piriform circuits that may differ from neocortical sensory areas.

Disclosures: **A.M. Oswald:** None. **A.M. Large:** None. **N.A. Kunz:** None. **S. Mielo:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Support: NIDCD - 1R01 DC014487-01A1

Pew Scholars

NARSAD Young Investigator Grant

Title: Anterior olfactory nucleus and piriform cortex feedback differentially modulate olfactory bulb output neurons

Authors: ***H. CHAE**[1], G. H. OTAZU[1], D. F. ALBEANU[1,2];
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Abstract: The olfactory bulb (OB) output neurons, the mitral and tufted cells project in a distributed fashion to several brain areas including the piriform cortex (PC) and anterior olfactory nucleus (AON). These, in turn, send massive glutamatergic projections back into the OB. We recently reported (Otazu, Chae, et al., Neuron, 2015) that pharmacological inactivation of the anterior piriform cortex (APC) increases odor responsiveness and pairwise similarity of mitral cells, but has little impact on tufted cells. Therefore, we proposed that PC feedback specifically acts on mitral cell representations to enable odor separation, while only mildly altering tufted cell responses. However, how feedback from AON controls these two classes of OB output neurons remains unknown to date. We used multiphoton calcium imaging (GCaMP) to monitor the odor responses of mitral and tufted cells in in awake head-fixed mice. Pharmacological inactivation of the AON increased odor responsiveness and pairwise similarity

of both mitral and tufted cells. We propose that feedback from PC and AON differentially modulate olfactory bulb output neurons. We are currently exploring optogenetic strategies to locally suppress distinct feedback signals to the bulb, and assess their contributions to olfactory behaviors.

Disclosures: H. Chae: None. **G.H. Otazu:** None. **D.F. Albeanu:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: R00 DC009839

Mallinckrodt Foundation

Title: How feedforward and feedback inhibition shape odor responses in piriform cortex

Authors: *K. A. BOLDING, K. M. FRANKS;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: We sought to understand how odor information is transformed from olfactory bulb to piriform cortex. We first obtained simultaneous recordings from populations of olfactory bulb mitral/tufted cells (M/Ts) and layer II principal cells in piriform cortex of awake, head-fixed mice. M/T activity increased in response to odor and remained elevated through the inhalation phase of the sniff. In contrast, piriform spiking showed only a small, transient odor response at inhalation onset, followed by marked suppression – as if the cortex were only responding to the earliest bulb inputs. We examined this transformation more directly using Thy1-ChR2 mice, which express channelrhodopsin (ChR2) in M/Ts, allowing us to precisely control the strength and duration of olfactory bulb output using light. Indeed, we found that sustained M/T spiking evoked a sharp but very brief increase in piriform spiking at light onset. This result indicates that inhibitory circuits within piriform cortex must truncate the cortical response to sustained bulb input. Principal cells in piriform receive inhibitory inputs from two distinct populations of inhibitory interneurons: feedforward interneurons (FFIs) in layer I, which receive excitatory inputs from M/Ts, and feedback interneurons (FBIs) in layer II/III, which receive excitatory inputs from principal cells themselves. To determine how these different types of inhibition shape cortical odor responses we next recorded from optogenetically identified GABAergic neurons in VGAT-ChR2 mice, which selectively express ChR2 in inhibitory interneurons. We

could distinguish between FFIs and FBIs by their laminar position relative to cell-dense layer II. Layer II neurons that did not show sustained light-evoked spiking were presumed to be principal cells. Again, population spiking in principal cells increased slightly and transiently at inhalation onset, followed by sustained suppression. Superficial (presumptive FFIs) and deep (presumptive FBIs) GABAergic neurons had strikingly different odor responses. Individual FFI responses were highly heterogeneous while FBIs were broadly responsive to most presented odors. Furthermore, FFI spiking increased slowly, peaked modestly, and exhibited a time course similar to the bulb odor response. In contrast, spiking in FBIs increased rapidly after inhalation, peaking ~10 ms after the peak of the principal cells' activity, and corresponding to the sharp onset of suppression in their population activity. These findings suggest that layer II/III FBIs play a dominant role in transforming odor representations in piriform cortex.

Disclosures: K.A. Bolding: None. **K.M. Franks:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: R00 DC009839

Mallinckrodt Foundation

Title: Coding strategies for representing odor identity and odor intensity in piriform cortex

Authors: *K. M. FRANKS, K. A. BOLDING;
Neurobio., Duke Univ., Durham, NC

Abstract: How can an animal accurately discriminate an odor at different intensities while still recognizing that odor's identity? The number and timing of odor responsive mitral cells may change with concentration but their activation sequences are typically concentration invariant. One model, proposed by Hopfield (1995) and elaborated by many others since, suggests that a downstream decoder that attends to the earliest bulb inputs could produce a largely concentration-invariant representation of odor identity. A corollary of this model is that odor intensity may be extracted from temporal structure of the response. We tested this model by recording from populations of piriform cortex neurons in awake, head-fixed mice following presentation of different odors at multiple concentrations. Our analyses focused on activity in the first full sniff after odor onset. Different odors (0.3% v/v) evoked distinct patterns of activity in

which ~10% of neurons increased spiking while ~15% of neurons were suppressed. Although responses exhibited considerable trial-to-trial variability, responses to repeated presentations were more similar (cc: ~0.5) than presentations of different odors (~0.35). These differences were sufficient for a linear classifier, using simple spike counts as input, to accurately identify the odor. Consistent with both Hopfield (1995) and Miura et al (2012), spike timing provided little additional information about odor identity. The number of both activated and suppressed cells increased with odor concentration so that total output was nearly constant across concentrations. Responses evoked by an odor at different concentrations were therefore similar but not invariant, and our linear classifier could accurately identify a familiar odor, even when presented at a novel concentration. However classifiers performed poorly when required to determine odor concentration. As predicted, responses peaked earlier with increasing odor concentration, and classifier performance at intensity discrimination improved markedly when temporal information was included. However, the Hopfield model predicts that information about odor identity and intensity should become available concurrently yet our classifier could rapidly decode identity ~100 ms before decoding odor intensity. This occurs because concentration-dependent latency changes were largely restricted to late-responding cells while the earliest responding cells were more concentration-invariant. We therefore suggest a revised two-stage decoding model in which a rapid representation of odor identity is formed and then refined to include information about stimulus intensity.

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Poster

527. Higher-Order Processing of Olfactory Stimuli

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Program#/Poster#: 527.15/UU10

Topic: D.04. Olfaction and Taste

Title: Neural relativity in dual networks

Authors: *A. KOULAKOV[1], D. KEPPLE[2], H. GIAFFAR[2], D. RINBERG[3];
[1]Cold Spring Harbor Lab., Cold Spg Hbr, NY; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [3]New York Univ., New York, NY

Abstract: Sensory systems are constantly facing the problem of computing the stimulus identity that is invariant wrt several features. In the olfactory system, for example, odorant percepts have to retain their identity despite substantial variations in concentration, timing, and background. This computation is necessary for us to be able to navigate in chemical gradients or within variable odorant plumes. How can the olfactory system robustly represent odorant identity

despite variable stimulus intensity? We propose a novel strategy for the encoding of intensity-invariant stimulus identity that is based on representing relative rather than absolute values of the stimulus features. We propose that, once stimulus features are extracted at the lowest levels of the sensory system, the stimulus identity is inferred on the basis of their relative amplitudes. Because, in this scheme, stimulus identity depends on relative amplitudes of features, identity becomes invariant with respect to variations in intensity and monotonous non-linearities of neuronal responses. For example, in the olfactory system, stimulus identity can be represented by the identities of p strongest responding odorant receptor types out of 1000. We show that this information is sufficient to ensure the robust recovery of a sparse stimulus (odorant) via l1 norm or elastic net loss minimization. Such a minimization has to be performed under the constraints imposed by the relationships between stimulus features. We map this problem onto a dual problem of minimization of a functional of Lagrange multipliers. The dual problem, in turn, can be solved by a neural network whose Lyapunov function represents the dual Lagrangian. We thus propose that the networks in the piriform cortex computing odorant identity implement dual computations with the sparse activities of individual neurons representing the Lagrange multipliers. Our theory yields predictions for the structure of olfactory connectivity.

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Poster

527. Higher-Order Processing of Olfactory Stimuli

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 527.16/UU11

Topic: D.04. Olfaction and Taste

Title: Correlated tuning within the olfactory tubercle

Authors: T. REDMOND, R. CHIRINOS, J. GOTTO, G. MINOGUE, C. TENUTO, *G. A. COUSENS;
Drew Univ., Madison, NJ

Abstract: Diffuse patterns of divergent and convergent projections from main olfactory bulb mitral and tufted cells contribute to an apparent lack of spatial topography in piriform cortex (PC), and evidence from studies using a range of techniques suggests that odor-selective responses of PC neurons are sparsely distributed and randomly organized. However, differences in the the topography and cellular composition of bulbar projections to other regions of the ventral telencephalon, as well as differences in the local circuit organization of these regions, suggests that features of odor representation across primary olfactory areas may be quite diverse. In particular, the finding that glomerular projections to cortical amygdaloid nuclei are patchy

suggests that olfactory tuning profiles may be spatially segregated to some extent in some olfactory areas. Here we examine correlated tuning within the olfactory tubercle (OT) using single- and multi-electrode recording techniques in urethane-anesthetized rats. Consistent with published reports, a large proportion of OT neurons exhibited odor-selective alterations in firing rate often in phase with ongoing respiration and local field potential oscillations. Cells exhibited a range of tuning breadths to molecularly distinct odorants, including monomolecular odorants and biologically significant pheromones, and adjacent cells were often similarly tuned. Preliminary evidence shows that interneuronal distance predicts the likelihood of correlated tuning between simultaneously recorded cells, suggesting a functional organization that differs from PC. Ongoing work using configurable high-density silicon probes positioned within OT and adjacent olfactory areas is examining the extent to which spatial and temporal features of odor-elicited neuronal activity compare directly across regions.

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Poster

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Topic: D.04. Olfaction and Taste

Support: This work is supported by the NIH/NIDCD grant to D.W. (R01DC014443)

Title: Optical stimulation of an olfactory corticostriatal pathway modulates odor coding in the olfactory tubercle.

Authors: *K. A. WHITE[1], D. W. WESSON[1,2];
[1]Biol., Case Western Reserve Univ., Cleveland, OH; [2]Neurosciences, Case Western Reserve Univ. Sch. of Med., Cleveland, OH

Abstract: How second-order sensory structures both inform and shape one another's neural activity is critical for understanding the way in which sensory information is encoded across hierarchical networks. In the olfactory system, axonal association fibers from the piriform cortex (PCX) innervate a number of secondary olfactory structures. One of these secondary structures, the olfactory tubercle (OT), is highly unique in comparison to all others given its position within the ventral striatum. The function of this PCX-OT corticostriatal connection is entirely unknown. Thus, the aim of this study is to elucidate whether, and if so how, PCX pyramidal cells modulate odor coding within the OT. To accomplish this, PCX pyramidal cells and their axon terminals

were transduced with a viral vector carrying humanized channelrhodopsin under control of the CamKIIα promoter. This approach transduced soma primarily in layer ii of the mouse PCX, whose association fibers were visible in all layers of the OT. Pyramidal cell bodies or, separately, axon terminals from the PCX were stimulated as we recorded single-unit neural activity from the OT of mice performing an olfactory task. We found that both stimulation of PCX neurons directly within the PCX, and stimulation of their axon terminals locally within the OT, robustly altered OT neuron spontaneous activity. In addition, the encoding of odors among OT neurons differed depending on the presence or absence of PCX stimulation. In some neurons, PCX stimulation during odor presentation had an additive effect on OT neuron firing rate. In contrast, among other neurons, PCX stimulation resulted in a subtractive effect upon odor responses. Thus, our results suggest that PCX input alters OT neuron ensemble activity in a way that is important for the encoding of olfactory information. Further, this work provides insights into schemes of information processing between secondary sensory structures.

Disclosures: **K.A. White:** None. **D.W. Wesson:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

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Program#/Poster#: 527.18/UU13

Topic: D.04. Olfaction and Taste

Support: NSERC Grant

Title: The smell of fear: rodent pheromonal communication of danger

Authors: ***S. GOODMAN**[1], I. T. K. MACINTYRE[2], Q. YUAN[2];
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Abstract: Pheromones are substances which can communicate a variety of messages between conspecifics. Alarm pheromones, widely studied in insects but not so much in mammals, serve to alert a member of the same species about impending danger. We aimed to investigate whether classically conditioned fear may be communicated across rats and thereby transferred to a conspecific in the absence of an external aversive stimulus. Male and female adult rats were either trained to associate a novel odor (terpinene) with a shock, or they were exposed to only shock or odor, not both. Odor exposed rats were either housed alone, or with an odor/shock conditioned rat. All animals were exposed to terpinene then a novel control odor octanol on the day following training, and freezing behavior to each odor was measured. Animals given shock

and odor showed a highly convincing trend towards increased freezing behavior specifically to terpinene compared to the controls, but more animals are needed to reach statistical significance. Animals exposed only to the odor and then housed with the conditioned animals showed more robust freezing behavior specific to the terpinene than either of the control groups. These results suggest that rats may be able to communicate their own specific fear memory to one another, perhaps via pheromones. Does pheromone mediated fear learning activate distinct neural circuities from those activated by conditioned fear? To investigate this question, cellular compartmental analysis of temporal activity by *in situ* hybridization will be employed. This technique utilizes immediate-early genes Homer1A (H1A) and Arc to visualize cells that are active to two temporally distinct events. H1A is expressed in the nucleus ~30 min following a stimulus, while Arc appears in the nucleus ~5 min after stimulus presentation. On the day following testing, rats will be exposed to octanol for 5 minutes, clean air for 20 minutes, terpinene for 5 minutes, then immediately sacrificed. Therefore, cells expressing H1A are those activated by the control odor octanol, while cells expressing Arc those are activated by the conditioned odor terpinene. A control odor is used to normalize each animal's response to the conditioned odor, providing intra-animal control. By mapping the cellular activation pattern across structures involved in olfactory fear conditioning in addition to those involved in pheromone detection, a clear picture will be visible regarding the process of this memory formation. We will systematically measure the H1A and Arc expression in the main olfactory bulb, accessory olfactory bulb, sub-regions of the piriform cortex, olfactory tubercle and several nuclei of the amygdala.

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Poster

527. Higher-Order Processing of Olfactory Stimuli

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Topic: D.04. Olfaction and Taste

Support: NIH RO1

HFSP Long-Term Fellowship LT001090

Title: Representation of odors in the postero-lateral cortical amygdala

Authors: *G. IURILLI, R. S. DATTA;
Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Olfactory information is conveyed from primary sensory epithelium in the nose to the main olfactory bulb (MOB) in the forebrain. After processing in the MOB, mitral/tufted cells broadcast olfactory information to several higher olfactory areas including the piriform cortex (PC) and the postero-lateral cortical amygdala (plCOA). The role of these distinct, parallel pathways however remains unclear. Mitral cells from the same glomerulus disperse their axons across the whole PC, but they send patchy and stereotyped projections to the plCOA. This differential patterning suggests that PC and plCOA perform distinct sensory transformations and therefore have distinct behavioral functions; consistent with this possibility, functional experiments have implicated the PC in associative learning whereas the plCOA has been suggested to participate to odor-driven innate behaviors. These anatomic and behavioral differences suggest dramatic differences in odor coding in these two olfactory cortices; however, the odor response properties of primary neurons in the cortical amygdala have not been characterized. Here, we employ multi-array, extracellular recordings to investigate the olfactory tuning properties of plCOA neurons in awake mice. We find that single neuron and population plCOA responses are similar to those observed in PC. Moreover, plCOA neurons were not better able to categorize aversive versus appetitive odors than neurons in the PC. These results suggest that intrinsic and feedback connectivity re-formats olfactory information in plCOA to generate odor representations that are similar to PC, suggesting that the plCOA may also play an important role in odor learning.

Disclosures: G. Iurilli: None. **R.S. Datta:** None.

Poster

527. Higher-Order Processing of Olfactory Stimuli

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 527.20/VV1

Topic: D.04. Olfaction and Taste

Support: NIH Grant DC014701

NIH Grant DC014367

Title: Mapping odor learning-dependent expression in FosTRAP mice using CLARITY

Authors: *S. LUO, K. XIE, J. R. AZARI, M. EINHORN, T. A. CLELAND;
Psychology, Cornell Univ., Ithaca, NY

Abstract: The direct comparison of appetitive and aversive forms of learning has been difficult with conventional training paradigms due to their substantial procedural differences. While

previous studies have examined immediate-early gene (IEG) expression following rewarded or fear learning, it is difficult to assess IEG responses to valence *per se*, owing to the procedural differences which exist between appetitive and aversive conditioning paradigms. Previously, we developed a novel olfactory training protocol for rats capable of evoking both forms of conditioning with minimal procedural differences, enabling outcomes to be directly compared using identical behavioral and physiological analyses. Using chronically implanted intra-oral cannulae, appetitive or aversive tastants (saccharin or quinine) were infused into the rats' mouths and paired (or backward-paired) with an odor conditioned stimulus (CS) such that training procedures differed only in the valence of the tastant. Rats displayed anticipatory behaviors (rapid mouth movements, tongue protrusions, gaping) in response to the odor CS predicting tastant infusions, indicating that they recognized the differing valences of the tastants and had learned the odor-tastant association. Using ultra thin-section immunohistochemistry, we have extensively mapped Egr-1 and c-Fos expression in olfactory- and valence-associated brain regions following appetitive and aversive conditioning, including olfactory bulb, anterior olfactory nucleus, anterior and posterior piriform, agranular insular, orbitofrontal, prelimbic/infralimbic, cingulate cortices, dorsal and ventral striatum, olfactory tubercle, basolateral amygdala, and hippocampus. We have quantified IEG expression along the rostrocaudal axis for a large number of subregions and sublayers, and have generated high-resolution histomicrographs illustrating their expression. Furthermore, we have quantified IEG expression along various axes in the anterior piriform and agranular insular cortices. Here, we continue to elucidate the neural networks involved in odor learning using the Targeted Recombination in Active Populations (TRAP) approach and CLARITY technique to visualize active neurons during learning in transparent brains. We use 4-hydroxytamoxifen (4-OHT) to induce recombination in FosTRAP (Fos (CreER/+) R26(AI14/+)) mice. The active cell population is identified via the expression of tdTomato (an effector gene). Subsequently, the FosTRAP brains are passively cleared using CLARITY techniques, and are imaged using light sheet (Ultramicroscope; LaVision BioTec) and confocal microscopy (Zeiss LSM 710).

Disclosures: S. Luo: None. **K. Xie:** None. **J.R. Azari:** None. **M. Einhorn:** None. **T.A. Cleland:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 528.01/VV2

Topic: D.05. Audition

Support: NSF Grant SMA-0835976

NIH/NIDCD Grant P50 DC013027

Title: Neural trajectory of auditory stream segregation across development

Authors: *S. SCHWARTZ[1], L. WANG[2], H. TAGER-FLUSBERG[3], B. SHINN-CUNNINGHAM[2];
[1]Grad. Program for Neurosci., [2]Biomed. Engin., [3]Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: Studies of how the brain perceptually organizes mixtures of acoustic signals and how this changes through development are important for understanding how children acquire language. Such studies will give us better insight into how children segment language from the mixture of sounds in their environment and how they learn to process spoken language in a world of complex auditory inputs. Through event-related potentials (ERPs) in adults, we can directly measure cortical responses to sounds and use this information to infer how those sounds are perceptually organized at early stages of auditory processing. However, research has yet to establish the extent to which we can measure equivalent cortical responses in young children. In this study we used a Mismatch Negativity Paradigm (the MMN) to explore passive, automatic processes governing auditory scene analysis in the young, developing brain. Specifically, we explored the degree to which spectral features of multiple, simultaneous sound streams affect sound organization in children. Children ages 3-18 passively listened to either one or two diotic sound streams while watching a silent movie with subtitles. Consistent with prior studies, we found that children of all ages exhibit sensitivity to unexpected sound events, or "deviants," occurring within a single stream of sound. When a second, higher-pitched interfering stream was presented simultaneously with the original stream, the deviant ERP response in children ages 6-18 looked similar to that present in adult listeners during similar perceptual segregation tasks. In contrast, children ages 3-5 do not produce a clear deviant ERP response in the dual-stream context. These results suggest that for children under age 6 (an age where children typically are entering formal learning environments), analysis of sound in a noisy environment cannot rely on the automatic processes that support perception in older children and adults. This work provides a foundation for comparing central auditory processing in typically developing young children with that of children with clinical disorders.

Disclosures: S. Schwartz: None. **L. Wang:** None. **H. Tager-Flusberg:** None. **B. Shinn-Cunningham:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 528.02/VV3

Topic: D.05. Audition

Title: Characterizing the evolution of cortical auditory evoked potentials and the influence of prepulse inhibition across decibel levels

Authors: T. POTTER[1], T. NGUYEN[1], S. LI[2], *Y. ZHANG[1];
[1]Univ. of Houston, Houston, TX; [2]TIRR Mem. Hermann Res. Ctr., Houston, TX

Abstract: Cortical auditory evoked potentials (CAEPs) are known, time-locked EEG potentials generated in response to acoustic stimuli. CAEPS Comprise primarily of the N1 and P2 peaks on EEG recordings. The presentation of CAEPs is noticeably altered by the addition of a non-startling prepulse, occurring between 50 and 350 ms prior to the focal sound. Despite their relevance and non-invasive observation, much remains unknown regarding how CAEPs evolve with varying stimulus intensity and how the presence of a constant prepulse changes this profile. In this study, 11 healthy subjects were recruited to perform an acoustic startle paradigm. Subjects were seating 12 inches in front of a JBL EON speaker, facing away. 64-channel EEG recordings (BrainVision Acticap) were performed with channels sacrificed to monitor the EKG and EMG in the Bicep, and Sternocleidomastoid muscle. The auditory paradigm consisted of a series of tasks featuring 20x 500 Hz sounds varied across 5 decibel levels (70, 80, 90, 100, and 110 dB), with and without a 70 dB prepulse occurring 50ms prior to the stimulus (10 tasks in total). Subjects were initially exposed to 5x 110 dB sounds without a prepulse to habituate the startle response, after which tasks alternated between prepulse (PP) and non-prepulse (NP) with randomized decibel levels. EEG signals from the Fz and Cz channels were processed (filtration, artifact rejection ICA, baseline correction), average peak amplitudes for the N1 CAEP component were ascertained for each subject. FFT analysis was also performed to examine the mean Theta oscillation activity. Main effects were determined using a two-way repeated-measures ANOVA (Decibel levels x5 and prepulse x2). Results showed a significant main effect of decibel levels on N1 amplitude across both Fz and Cz channels. Further analysis showed that the presence of a prepulse significantly increased the N1 amplitude at 70 dB of stimulus pulse while decreasing it at 90 and 110 dB in both the Fz and Cz channels, with significant decrease also observed in the 100 dB condition at Cz. Similarly, a significant main effect of decibel levels was shown on theta activity in the Cz electrode. The prepulse significantly increased the theta activity in the 70 dB condition and decreased at 110 dB in both Fz and Cz channels, with 80 db also showing a significant increase at Cz. These results indicate a clear evolution of the CAEP in response to stimulus intensity without a prepulse. In the presence of a prepulse, the CAEP response depends

on the interactions between the prepulse and the stimulus, arguing against the interruption or protection hypothesis of prepulse inhibition.

Disclosures: **T. Potter:** None. **T. Nguyen:** None. **S. Li:** None. **Y. Zhang:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 528.03/VV4

Topic: D.05. Audition

Support: NIH 2R01DC05660

Title: Temporal information recovered from speech fine structure contributes to understanding temporally distorted speech: psychophysics and MEG evidence

Authors: ***X. TENG**[1,2], D. POEPPEL[3,2];
[1]Dept. of Psychology, [2]New York Univ., New York, NY; [3]Max Planck Inst., Frankfurt, Germany

Abstract: The speech envelope is often considered to be the most important temporal cue for speech perception, and the auditory system relies on the speech envelope to extract acoustic information on different timescales. Here, we show that temporal fine structure improved speech intelligibility of temporally distorted speech and entrained cortical oscillation, by supplying envelope information. We first distorted the temporal structure of speech by locally reversing speech segments and evaluated the contribution of the temporal fine structure to intelligibility. The temporal fine structure was found to help listeners restore critical temporal information of speech. We then used a method not yet widely appreciated, cochlear scaled correlation, to derive temporal information from the temporal fine structure. Using MEG and mutual information analysis, we evaluated whether the derived temporal information can entrain cortical oscillations and correlate with the original envelope of speech depending on the number of cochlear bands used. Our findings emphasize the importance of temporal fine structure in speech perception and invites us to rethink the canonical view on the relationship between amplitude envelope and temporal fine structure.

Disclosures: **X. Teng:** None. **D. Poeppel:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

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Topic: D.05. Audition

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Title: Joint tuning to sound features emerges in superficial layers of human primary auditory cortex

Authors: *M. MOEREL[1,2,3], F. DE MARTINO[2,3], K. UGURBIL[3], E. YACOUB[3], E. FORMISANO[1,2];
[1]Maastricht Ctr. for Systems Biol. (MaCSBio), [2]Dept. of Cognitive Neurosci., Maastricht Univ., Maastricht, Netherlands; [3]Ctr. for Magnetic Resonance Res., Univ. of Minnesota, Minneapolis, MN

Abstract: Electrophysiological recordings in animal models[1,2] suggest there may be relevant transformations in sound processing taking place between middle primary auditory cortical (PAC) layers (receiving thalamic input) and superficial PAC. Here we use ultra-high field fMRI (7 Tesla) to measure and compare sound responses at deep, middle and superficial cortical depths of human PAC. We acquired high-resolution anatomical (0.6 mm isotropic) and functional data (0.8 mm isotropic), while volunteers (N = 6) listened to 144 natural sounds. We used the anatomical data to define cortical depths and identify the PAC based on myelin-related contrast at individual level. Cortical responses were analyzed inside and outside PAC with three encoding models, representing different hypotheses on sound processing. A first *tonotopy* model was simplest, describing sound processing by frequency preference. A second *independent modulation* model hypothesized, in addition to frequency, preference to temporal and spectral modulations. A third *joint modulation* model was most complex, describing sound processing as the frequency-specific tuning of neuronal populations to combined spectral and temporal

modulations[3,4]. In the PAC only, we observed a significant interaction such that the difference in model prediction accuracy varied with cortical depth (RM ANOVA; p = 0.006). Only at a superficial cortical depth, the *joint modulation* model performed (marginally) significantly better than the other two models (p(corrected) = 0.02 and 0.07 for the *tonotopy* and *independent modulation* model, respectively). In non-primary auditory regions, the difference in model performance did not vary with cortical depth (no significant interaction; main effect of 'model'; p =0.003). Paired t-tests confirmed that the *joint modulation* model significantly outperformed the other two models (p(corrected) = 0.02 and 0.001 for the *tonotopy* and *independent modulation* model).In accordance with previous invasive animal studies[1,2], these results suggest the emergence of joint tuning to sound features in superficial PAC. That is, while neuronal populations in middle PAC layers are tuned independently to distinct sound features, neuronal populations in superficial PAC encode specific feature combinations. Joint tuning is propagated throughout cortical depths in non-primary auditory cortex, and may be a first computational step for forming a complex representation of the physical input towards sound abstraction and perception.1. Sadagopan & Wang (2009) J. Neurosci; 2. Sharpee et al. (2011) Curr. Opin. Neurobiol. 3. Chi et al. (2005) JASA; 4. Santoro et al. (2014) PLoS Comp Biol.

Disclosures: **M. Moerel:** None. **F. De Martino:** None. **K. Ugurbil:** None. **E. Yacoub:** None. **E. Formisano:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 528.05/DP05 (Dynamic Poster)

Topic: D.05. Audition

Support: Internal grants from the Cognitive and Behavioral Neuroscience Unit at IDOR

Title: "Name that tune": identifying musical pieces from fMRI data using encoding and decoding models

Authors: *S. HÖFLE[1,2], A. ENGEL[3,1,4], R. BASILIO[1], V. ALLURI[5,6], P. TOIVIAINEN[5], M. CAGY[2], J. MOLL[1];
[1]D'Or Inst. for Res. and Educ. (IDOR), Rio de Janeiro, Brazil; [2]Biomed. Engin. Program, COPPE, Federal University of Rio de Janeiro, Brazil; [3]Clin. for Cognitive Neurol., University Hospital Leipzig, Germany; [4]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [5]Finnish Ctr. for Interdisciplinary Music Research, Dept. of Music, University of Jyväskylä, Finland; [6]Neurosci. of Emotion and Affective Dynamics Lab, Swiss Ctr. for Affective Sciences, Biotech Campus, Geneva, Switzerland

Abstract: Introduction: The neural correlates of listening to music have been investigated in several ways (Koelsch, 2014). However, mapping ongoing brain activity during naturalistic music listening combined with detailed models of musical features is an emerging approach (Alluri et al. 2012, 2013, Toiviainen et al. 2013). The so-called "encoding models" allow capturing the effects of multiple stimulus variables on brain responses that can be used subsequently to decode or identify stimuli from brain activity (Naselaris et al. 2011). Here we apply this new concept of combining encoding/decoding models in order to identify musical content from brain activity. **Method:** We scanned 6 subjects on 4 different days on a Philips Achieva 3T scanner. Blood oxygen level dependent (BOLD) signal was measured with a T2* weighted EPI sequence while the subjects listened to 40 music pieces of different genres (total listening and fMRI scanning time: 2 h 8 min per subject across 4 days). Then, we modeled the individual brain responses in the auditory cortex and surrounding regions (superior and middle temporal gyri) by means of a multiple linear regression using 21 musical features (comprising rhythmic, timbral and tonal features, see Alluri et al. 2012). During the encoding phase, the coefficients were estimated in a leave-one-out scheme, while always two music pieces of 46 s were excluded for determining the coefficients. During the decoding phase, we identified which one of the two pieces was more likely to predict the measured BOLD signal (Stansbury et al. 2013). Identification accuracy was then determined as the percentage of correctly and incorrectly identified pieces. **Results:** For all 6 subjects identification accuracies significantly above chance level were obtained (76.8% ± 6.5%, mean ± sd of best accuracy across subjects). Accuracy levels increased both with number of voxels and time points, as expected. More specifically, accuracy levels increased linearly with time, whereas the relationship between number of voxels and accuracy was marked by a steep increase from 2 to 50 voxels but then stabilized from 50 to 300 voxels. **Conclusion:** In conclusion, in this study, we highlighted the spatio-temporal relationship of encoding/decoding models. The present work extends previous studies that focused either on encoding models (Alluri et al. 2013) or decoding models (Toiviainen et al. 2013) alone. Our results showed the importance of the combination of multivariate analysis with encoding models to improve music identification accuracy.

Disclosures: S. Höfle: None. **A. Engel:** None. **R. Basilio:** None. **V. Alluri:** None. **P. Toiviainen:** None. **M. Cagy:** None. **J. Moll:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 528.06/VV6

Topic: D.05. Audition

Support: NWO VIDI 864-13-012

Title: Investigating the functional organisation of the human auditory pathway with high-resolution fMRI

Authors: *F. DE MARTINO[1], O. GULBAN[2], E. FORMISANO[2];
[1]Fac. of Psychology, Maastricht Univ., Maastricht, Netherlands; [2]Cognitive Neurocience, Maastricht Univ., Maastricht, Netherlands

Abstract: Understanding how the human brain extracts behaviorally relevant information from sounds necessitates mapping sub-cortical and cortical processing levels simultaneously. So far, technical challenges have limited the coverage and/or resolution of functional magnetic resonance imaging (fMRI) studies of the human auditory pathway [1-3]. Here we investigate the topographic organization of the auditory pathway from the cochlear nucleus to the auditory cortex at high spatial resolution. We collected anatomical (0.7 mm isotropic) and functional data (1.1 mm isotropic) at 7 Tesla. During functional imaging participants ($N = 6$) listened to natural sounds (1 s) presented in the silent gaps. FMRI encoding [4] was used to analyze the functional responses to natural sounds and investigate the spatial organization of preferences to acoustic properties for every subject independently. The consistency of the results across subjects was evaluated in a common cortical space (cortex based alignment) and a common sub-cortical space obtained through non-linear registration (FNIRT-FSL) of the anatomical data. A preliminary analysis of allowed identifying, in every subject, sub-cortical and cortical regions with a significant (FDR corrected q=0.05) response to the sounds (see Figure 1). Cortical frequency preference showed tonotopic maps in accordance with previous studies [1]. These results show that high-field fMRI can be used to investigate the functional organization of the entire human auditory pathway at a resolution sufficient to highlight the topographic organization of acoustic features across all stages. The use of natural sounds and fMRI encoding will allow tracking the transformation of sound representations throughout the brain.
References. 1.Formisano, E*., et al.*,2003, Neuron. 2.Guimaraes, A.R*., et al.*,1998, Human Brain Mapping. 3.De Martino, F*., et al.*,2013, Nat Commun. 4.Moerel, M*., et al.*,2012, Journal of Neuroscience.

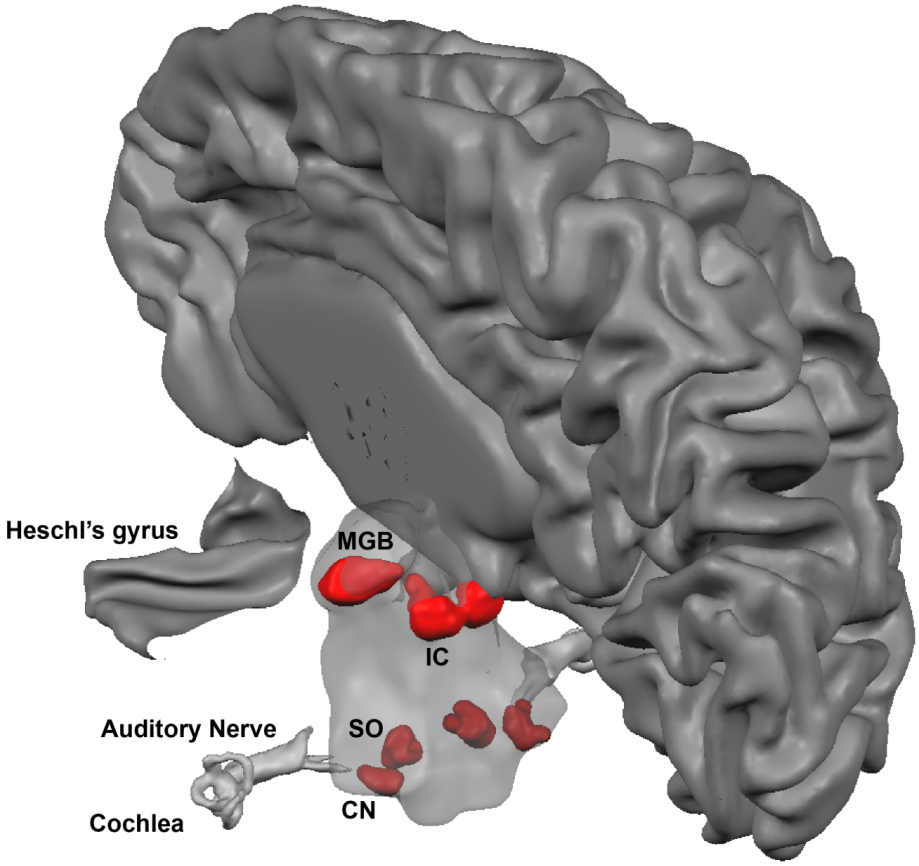

Figure 1. Single subject rendering of the auditory pathway. The cochlea and auditory/vestibular nerve are segmented from the anatomical images. Red areas indicated sub-cortical regions (cochlear nucleus [CN], superior olive [SO], inferior colliculus [IC] and medial geniculate body [MGB]) with significant (FDR corrected) response to natural sounds.

Disclosures: F. De Martino: None. **O. Gulban:** None. **E. Formisano:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 528.07/VV7

Topic: D.05. Audition

Support: Grant-in-Aid for Young Scientists (B) (15K19735)

Title: Longitudinal change in gamma band auditory steady-state responses in patients with schizophrenia

Authors: ***N. ORIBE**[1,2], H. KUGA[2,1], Y. HIRANO[1], H. TAKAO[2,1], T. ONITSUKA[1], T. UENO[2,1];
[1]Kyushu Univ., Fukuoka, Japan; [2]Natl. Hosp. Organization Hizen Psychiatric Ctr., Yoshinogari, Japan

Abstract: Background
Gamma band auditory steady state response (ASSR) have been reported to be abnormal and considered to be a robust biomarker in schizophrenia patients. However, the ASSR has been never investigated longitudinally in such patients as far as we know.
Methods
We measured ASSR responses at Baseline in schizophrenia patients (mostly in acute phase, SZ, N=6), as well as healthy control subjects (HC, N=7) while presenting click trains varying in rate of stimulation (20, 30, 40 and 80 Hz) and repeated the measurements after 3-15 months intervals (Follow up). EEG-evoked power and phase locking were obtained in response to each stimulation frequency.
Results
SZ showed significant reduction in phase locking to the 40Hz stimuli compared with HC at Baseline but the difference lost significance at Follow up. Interestingly, improvements of the symptom evaluated by Brief Psychiatric Rating Scale were associated with the PLF improvements in the patients.
Conclusions
These results suggest that neural circuits abnormalities indexed by the ASSR change dynamically in the course of the illness and the ASSR has the potential to be used as a marker for treatment response to antipsychotic drugs.

Disclosures: **N. Oribe:** None. **H. Kuga:** None. **Y. Hirano:** None. **H. Takao:** None. **T. Onitsuka:** None. **T. Ueno:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 528.08/VV8

Topic: D.05. Audition

Support: German Research Foundation - SPP1608

Title: Neural correlates of categorical auditory preprocessing of voice onset time in the brainstem

Authors: *A. BURGHARD[1,2], M. B. VOIGT[2], A. KRAL[2], P. HUBKA[2];
[1]Neurosci., UConn Hlth., Farmington, CT; [2]Inst. of Audioneurotechnology & Dept. of Exptl. Otology, ENT Clinics, Hannover Med. School, Germany, Hannover, Germany

Abstract: Detection of temporal sequences is crucial in auditory object recognition and categorization. This function of the auditory system allows phonetic analysis and determines speech understanding. The voice-onset time (VOT) is considered to be a parameter for categorization of voiced and unvoiced phonemes, but its neural correlates remain elusive. As shown in behavioral studies, this phonetical category is perceived similarly in animals and in humans. Therefore, its neurological substrates can be studied in animal models.In the present study, short and long noise bursts were used to model VOT in guinea pigs. The auditory brainstem response (ABR) was recorded to the initial noise burst (NB1, duration of 5-100 ms) and the trailing noise burst (NB2, duration of 50 ms), separated by a short gap (2-10 ms). All stimulus combinations were presented 600 times (300x each polarity) at 30 dB above hearing threshold. The ABR onset response to NB1 and NB2 was compared. The individual components (amplitude, latency, inter-peak-latency) were subsequently analyzed. In order to evaluate the general morphology of the onset ABR response, a 6 ms time window of the onset response was evaluated in the time domain (computation of RMS value and direct comparison of ABR waveforms). In addition, the time-frequency representation of the response was computed to assess speed and synchronization of the activation of the brainstem structures.In accordance with previous studies, a longer duration of the initial NB1 (30-100 ms) resulted in significantly weaker and delayed responses to the trailing NB2. However, the combination of a short initial NB1 (<30ms) with a gap duration of 10 ms significantly facilitated the onset NB2 response in the strength and speed of the sequence for ABR components II-IV (latencies of 2-5ms). In the latter stimulus combination, it is likely that the initial NB1 activation primed the brainstem structures resulting in a highly synchronized and rapid initial activation sequence. We propose that the described findings represent neural correlates of early phases of categorical auditory preprocessing in the auditory system, which might play an important role for the final categorization in the cortical structures.

Disclosures: **A. Burghard:** None. **M.B. Voigt:** None. **A. Kral:** None. **P. Hubka:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 528.09/VV9

Topic: D.05. Audition

Title: A system identification approach for rapid characterization of the auditory brainstem response (ABR)

Authors: D. P. DRENNAN, *E. C. LALOR;
Trinity Col. Dublin, Dublin, Ireland

Abstract: The event-related potential (ERP) has long been the canonical technique for conducting electrophysiological analyses of the human auditory system, and for good reason. As is apparent from the auditory brainstem response (ABR), ERPs can index processing along the brainstem with excellent temporal resolution. One shortcoming of the traditional ERP approach however is the relatively time-consuming nature of its acquisition, which can limit its clinical utility. An alternative approach which seeks to circumvent these limitations focuses on deriving what is known as a Temporal Response Function (TRF; Theunissen et al., 2001; Lalor et al., 2009). Typically this is done by regressing neural data against some feature of a continuous stimulus. In the context of EEG/MEG this has been done, for example, by regressing the data on each electrode against the envelope of an amplitude modulated carrier signal (Lalor et al., 2009), or even speech (Lalor & Foxe, 2010; Ding & Simon, 2012). This has been shown to elicit ERP style responses and, due to the continuous nature of the stimuli being presented, has facilitated research which could not easily be conducted using a traditional ERP approach (Power et al., 2012). However, the SNR of these TRFs is typically not as high as those of a traditional ERP. Also, it is unknown whether the approach can facilitate the resolution of ABR components. Here, we present a novel approach to deriving EEG TRFs using a stimulus that consists of a number of impulses which are pseudorandomly spaced, which we refer to as a 25% jittered (25PJ) click train. We characterize the components of these TRFs through comparison with ABRs derived using standard approaches. This novel stimulus paradigm could be useful in many subdomains of both clinical and cognitive auditory neuroscience research.

Disclosures: **D.P. Drennan:** Other; PhD part-funded by Neuromod Devices Ltd. under the Irish Research Council Enterprise Partnership Scheme. **E.C. Lalor:** Other; PhD part-funded by Neuromod Devices Ltd. under the Irish Research Council Enterprise Partnership Scheme.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 528.10/VV10

Topic: D.05. Audition

Support: National Centre of Science in Poland Grant, number: UMO-2013/11/B/HS6/01242

Title: The role of auditory steady-state responses in differentiating states of arousal.

Authors: *U. GÓRSKA, ESQ[1,2], M. BINDER[1], M. WYCZESANY[1];
[1]Psychophysiology Labolatory, Jagiellonian Univ., Krakow, Poland; [2]Neurophysiol. Labolatory, Donders Inst. for Brain, Cognition and Behaviour, Nijmegen, Netherlands

Abstract: Objective: Research on auditory steady-state responses (ASSRs) has demonstrated that this kind of evoked EEG activity is sensitive to the actual level of arousal in both NREM sleep (Tlumak et al., 2012) and general anaesthesia (Plourde, 1996). The aim of present study was to evaluate the strength and information transmission after periodic ASSR stimulation with broad range of modulation frequencies during deep NREM sleep and wakefulness.
Methods: In the present study 17 healthy awake and asleep (N2 and N3 stages) subjects were presented with series of auditory stimuli (carrier frequency 1000Hz) amplitude-modulated (AM) by the set of frequencies (4Hz, 6Hz, 8Hz, 12Hz, 20Hz, 40Hz) while measuring their brain activity with 64-channels EEG system. We estimated ASSR amplitude with noise-subtracting average and intertrial phase variability with squared phase coherence (PC) index. The suprathreshold cluster test ($p<0.05$) was used for the selection of electrodes that maximize differences between states. Moreover, we quantified the direction and intensity of information flow for sleep and wakefulness with Directed Transfer Function (DTF).
Results: The analysis of ASSR amplitudes revealed significant differences for 4Hz, 6Hz and 8Hz, with substantial amount of individual variability for other modulation frequencies. In turn, the PC parameter displayed effect of subject state for all presented modulation frequencies beside 12Hz (this lack of effect might be caused by sleep spindles activity). Correspondingly, for this frequency DTF did not reveal any sleep-wake differences. More importantly, in awake state DTF analysis demonstrated transmission of information from centro-parietal to frontal brain regions in the beta frequency range for lower modulation frequencies and 40Hz. These effects were absent in NREM sleep.
Conclusion: Summing up, state-dependent AM ASSR effects vary with modulation frequency. Specifically, results for PC and DTF measures suggest that 4Hz, 6Hz, 8Hz and 40Hz remain sensitive to the level of arousal. This demonstrates a key role of phase variability of ASSR responses in determining the neuronal integrity in various states of consciousness. Furthermore, our results suggest that long-range information flow accompanying ASSRs becomes suspended during NREM sleep unconsciousness. Taken together these measures open a possibility for reliable distinction between states of altered arousal e.g. in patients after severe brain damage who may not be able to move or communicate while retaining the capacity for conscious processing.

Disclosures: **U. Górska:** None. **M. Binder:** None. **M. Wyczesany:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 528.11/VV11

Topic: D.05. Audition

Support: NIH,NIDCD, DC014279

Pew Charitable Trusts, Pew Biomedical Scholars Program

Title: Sequential encoding of acoustic features in EEG responses to continuous speech

Authors: *B. KHALIGHINEJAD, G. CRUZATTO DA SILVA, N. MESGARANI; Columbia Univ., New York, NY

Abstract: When listening to speech, humans have the ability to simultaneously extract both the content of the speech and the identity of the speaker. This is due to a multitude of cues encoded in the acoustic signal, including distinctive features of phonemic categories that carry meaning, and identifiable features of the speaker such as pitch, prosody and accent. Recent findings from invasive human electrophysiology studies have shown the role of phonetic features in organizing the representation of speech sounds at higher level speech cortices. However, it remains unclear how the neural representation of acoustic features unfolds over time as the speech sounds propagate through the auditory pathway, and how linguistic and non-linguistic information are jointly encoded in the brain.

We recorded EEG data from 22 native speakers of American English. Participants listened to simple stories comprised of alternating sentences uttered by two speakers (one male, one female). We applied a novel analysis to electroencephalography (EEG) signals in response to continuous speech to characterize the neural representation of acoustic features and the progression of responses over time. By averaging the time-aligned neural responses to phoneme instances, we calculated the phoneme-related potential (PRP) and studied the joint representation of linguistic (phonetic) and non-linguistic (speaker) information in the PRPs. We observed four sequential time intervals during which the PRPs are organized by the acoustic similarity of phonetic categories, appearing approximately at 50 ms, 120 ms, 230 ms, and 400 ms relative to the phoneme onset. The responses are primarily organized by phonetic feature, while subtler speaker variations appear within manner groups. This is consistent with previous studies that have shown a larger role for phonetic over speaker characteristics in shaping the acoustic properties of phones. Additionally, the different scalp distributions at each time interval suggest a different underlying pattern of neural activity for each component.

A major difference between our study and previous work is that it provides a direct link between the organization of neural responses and the acoustic properties of speech sounds. Therefore, this study lays the groundwork for several research directions where explicit changes in the

representational properties of speech can be examined in speech development, as a listener learns new acoustic distinctions, second language acquisition, and through varying task demands.

Disclosures: **B. Khalighinejad:** None. **G. Cruzatto da Silva:** None. **N. Mesgarani:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

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Topic: D.05. Audition

Support: Dutch Province of Limburg

NWO VICI grant 453-12-002

NWO VENI grant 451-15-012

Title: A computational model of temporal processing in human auditory cortex

Authors: *I. ZULFIQAR, M. MOEREL, P. DE WEERD, E. FORMISANO;
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Abstract: Temporal information in sounds is relevant for many general purpose and domain specific auditory functions, including amplitude modulation (AM) detection, extraction of temporal pitch and decoding of speech. Each of these functions has been extensively studied with a variety of methods (electrophysiology, non-invasive neuroimaging, psychophysics). The common neural coding mechanisms underlying these observations, however, remain unclear. Here we propose a computational framework to integrate available empirical information to derive a unified view of temporal information processing in human auditory cortex (AC).
We designed a simplified model of the AC consisting of four functional units. These units approximately correspond to two core (AI, R) areas and two belt "streams" (Fast, Slow). Each unit was simulated using the Wilson Cowan Cortical Model (WCCM)[1] of neural circuitry. The WCCM generates dynamic neural responses by interaction of excitatory (E) and inhibitory (I) populations. AI and R receive cochleotopic thalamic input, the belt streams receive cochleotopic input from AI (Fast) and R (Slow) respectively. We adjusted the main parameters of the four units, temporal and spectral integration windows, based on physiological evidence from monkeys[2,3] and recent human fMRI[4].
First, we explored the model's coding of AM. For both noise and tones (0.125-8 kHz), we observed a switch from a temporal to a rate code for modulation rates above 50 Hz. The upper limit of temporal (but not rate) code was unit dependent, in accordance with electrophysiology[2].

Interestingly, estimated modulation transfer functions followed psychophysical modulation detection thresholds[5]. Also, we observed a dependence of AM coding on carriers in agreement with psychophysics[5]. Second, we tested how this same model represents temporal pitch[6], with missing fundamental stimuli and iterated ripple noise (IRN). AI and Fast temporally decoded low (<300 Hz) missing fundamentals while the Slow area represented high frequencies as phase coherence across the network. IRN was coded temporally with comparative weaker strength matching its weaker pitch percept.
Using a simple network of E and I interactions and tuning of only two parameters, we modeled processing of temporal information in parallel cortical streams. Readout of population responses are in agreement with human psychoacoustics. Future use of the model, in particular, to simulate response to speech is planned.
Footnotes
[1] Wilson and Cowan 1972 *Biophys. Journal*
[2] Bendor and Wang 2008 *J. Neurophysiol*
[3] Steinschneider et al. 1980 *Brain Research*
[4] Santoro et al. 2014 *PLOS Comp Bio*
[5] Moore 2012 *Brill*
[6] Bendor et al. 2012 *J Neurosci*

Disclosures: I. Zulfiqar: None. **M. Moerel:** None. **P. De Weerd:** None. **E. Formisano:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

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Topic: D.05. Audition

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NSF Grant CRCNS-1515678

The Hoover Fund

Title: Electrocorticographic (ECoG) delineation of human auditory cortical fields based on effects of propofol anesthesia

Authors: *K. V. NOURSKI[1], M. M. TODD[1], M. STEINSCHNEIDER[2], M. I. BANKS[3], A. E. RHONE[1], R. N. MUELLER[1], H. KAWASAKI[1], M. A. HOWARD, III[1];

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Abstract: Functional organization of human auditory cortex remains poorly defined. While the posteromedial portion of Heschl's gyrus (HG) is considered part of core auditory cortex, the presence of any further subdivisions remains unknown. Anterolateral HG has been variously considered to be part of core and/or surrounding belt. Propofol, used for induction of general anesthesia, affects auditory cortical activity (e.g. Plourde et al, Anesthesiology 2006 104:448-57; Davis et al, PNAS 2007 104:16032-7). This study tested the hypothesis that auditory cortical fields could be characterized by their differential sensitivity to propofol. Subjects were neurosurgical patients undergoing removal of ECoG electrodes placed to identify locations of epileptic foci. All procedures were approved by the University of Iowa Institutional Review Board. Each subject gave informed consent for this study. Stimuli were 50 Hz click trains (0.5 s duration, 1.5 s interstimulus interval), presented continuously during a baseline period and induction of anesthesia with stepwise increases in propofol infusion rate. ECoG recordings were made simultaneously from HG and superior temporal gyrus (STG) using depth and subdural grid electrodes, respectively. Anesthesia depth was monitored using spectral entropy (Viertiö-Oja et al, Acta Anaesthesiol Scand 2004 48:154-61). Averaged evoked potentials (AEP), frequency-following responses (FFR) and high gamma (70-150 Hz) event-related band power (ERBP) were used to characterize auditory cortical activity. Two distinct patterns were identified in posteromedial HG. In its most posteromedial aspect, earliest AEP deflections were preserved, and FFR increased during induction. The remainder of posteromedial HG exhibited attenuation of both the AEP and FFR. Anterolateral HG exhibited weaker activation; responses here were characterized by the lack of FFR and the presence of broad, low-voltage AEP off-response during induction. Lateral STG exhibited FFR that dissipated during induction. High gamma ERBP decreased during induction in all areas. Changes in auditory cortical activity did not always parallel changes in entropy. Differential patterns of auditory cortical activity during propofol induction are useful physiological markers for field delineation. Posteromedial HG is not uniform in its basic response properties and can be parcellated into two subdivisions. Preservation of the earliest AEP deflections and FFR likely reflects synaptic activity initiated in the medial geniculate body. Presence of FFR on the lateral STG suggests that portions of the lateral STG reflect a relatively early stage in the auditory cortical hierarchy.

Disclosures: K.V. Nourski: None. **M.M. Todd:** None. **M. Steinschneider:** None. **M.I. Banks:** None. **A.E. Rhone:** None. **R.N. Mueller:** None. **H. Kawasaki:** None. **M.A. Howard:** None.

Poster

528. Auditory Processing: Temporal and Frequency in Humans

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 528.14/VV14

Topic: D.05. Audition

Title: Using auditory brainstem responses to measure hearing loss induced increases in neural gain and its implications with tinnitus

Authors: *A. J. HARDY, J. DE BOER, K. KRUMBHOLZ;
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Abstract: The homeostatic plasticity model posits that tinnitus is triggered by an increase in neural gain due to a reduced input from a damaged periphery. Evidence for this mechanism in humans has come from auditory brainstem responses (ABR). ABRs recorded from tinnitus patients were found to show a reduced wave I amplitude but a normal wave V (Schaette 2011). This was interpreted as evidence of an increase in neural gain between the auditory nerve (wave I) and the upper auditory brainstem (wave V). However, a confound in this interpretation is that wave I is more dependent on contributions from high-frequency cochlear regions than wave V. High-frequency cochlear regions are also more affected by hearing loss. As a result, hearing loss may be expected to affect the wave I amplitude more than wave V, and this could be the driving factor behind the increased wave I/V ratio. Here, we aim to address this confound by measuring ABRs from restricted cochlear frequency regions.
Frequency-specific ABRs are obtained by restricting the response using high-pass masking noise with a variable cutoff frequency. Responses for different cutoff frequencies are subtracted to derive a response that only contains contributions from a specific cochlear region. This method has been used successfully to obtain frequency-specific click-evoked wave V responses. However, relative to wave V, the click-evoked wave I requires a high stimulus level to obtain an acceptable response. This means that uncomfortably loud HP noise would be required to obtain frequency-specific wave I responses. Furthermore, click stimuli are not optimal for eliciting wave I at high stimulus levels.
In this study we aim to address these problems by measuring chirp-evoked ABRs. We will use a rising chirp stimulus which starts at a low frequency and rises to a high frequency. Rising chirps have been used successfully in previous studies to increase the SNR of wave V (Fobel, 2004), as they compensate for the cochlear travelling-wave delay, causing the auditory nerve fibers to fire simultaneously and create a larger ABR. To further optimise frequency-specific chirps, we will use chirps that are weighted towards low-frequencies.
Once the optimal paradigm for measuring frequency-specific ABR wave I responses has been developed, we will measure the wave I/V ratio in normal hearing, tinnitus and matched hearing-loss groups. If there is a neural gain increase we will expect to see an increase in wave I/ V ratio

in hearing loss regions in the tinnitus group compared to normal/hearing matched groups but not in regions where hearing is intact.
Fobel & Dau(2004). JASA, *116*(4), 2213. doi:10.1121/1.1787523
Schaette (2011) J. Neurosci. 31(38), 13452–13457

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Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

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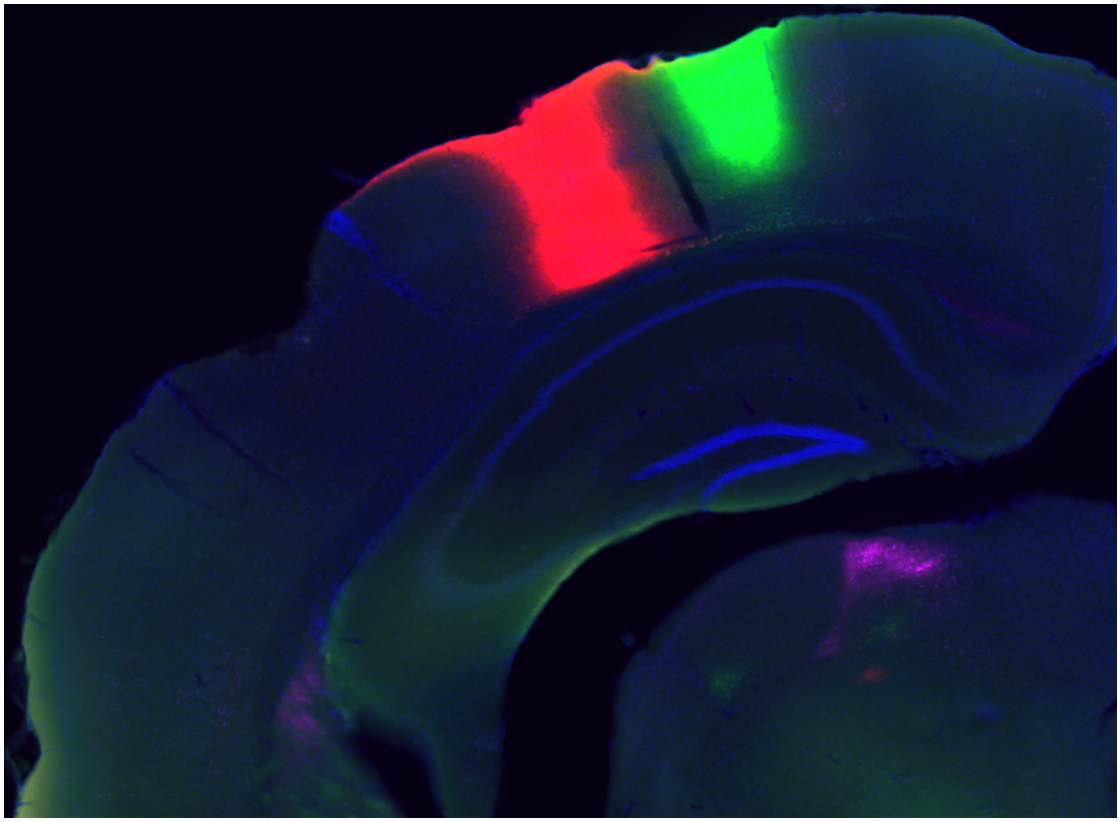
Title: A precise connectivity map between the mouse thalamic nucleus LP and visual cortical areas

Authors: *A. L. JUAVINETT[1], E. J. KIM[2], H. K. COLLINS[3], E. M. CALLAWAY[2];
[1]The Salk Inst., LA Jolla, CA; [2]The Salk Inst., La Jolla, CA; [3]UC San Diego, La Jolla, CA

Abstract: The visual thalamus is critical hub for incoming sensory information, though the role of the abundant feedforward and feedback connectivity between the thalamus and cortex remains elusive. Primate pulvinar is topographically and functionally organized, but a more granular map is needed to understand the role of thalamocortical loops in visually guided behavior. Similarly, the secondary visual thalamic nucleus in mice (LP) has extensive connections with cortex that are tractable with novel viral techniques. To resolve the precise connectivity of these circuits, we first mapped mouse visual cortex using intrinsic signal optical imaging and then injected a retrograde tracer (cholera toxin subunit B) into six different visual areas. We find that LP has separate zones that project to specific extrastriate regions, with few cells projecting to multiple visual areas. Additional experiments will investigate the sources of cortical and subcortical inputs to LP cells with known extrastriate projection targets using a rabies tracing approach. Disentangling these circuits will yield important insight into the role of the secondary visual thalamus in sensory processing as well as guide future functional studies.



Disclosures: **A.L. Juavinett:** None. **E.J. Kim:** None. **H.K. Collins:** None. **E.M. Callaway:** None.

Poster

529. Subcortical Visual Pathways

Location: Halls B-H

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Title: Direct projection of retinal ganglion cells to mesencephalon Kölliker-Fuse nucleus

Authors: *H. LU[1], L. ZHANG[2], Z. ZHAO[2], M. TAN[2], K. CHEN[2], K.-F. SO[2], C. REN[2];
[1]Guangdong-Hongkong-Macau Inst. of CNS Regeneration, [2]Jinan Univ., Guangzhou, China

Abstract: The neural connectivity between retinal ganglion cells (RGCs) and various mesencephalon regions including lateral geniculate nucleus (LGN) has been comprehensively described. The current anatomical knowledge, however, cannot fully explain the non-imaging function of RGCs. Advanced neural tracing tools are thus required to elaborate the network of RGC projection. In this study, we used CTB as the anterograde tracing marker via intraocular injection, coupled with immunofluorescence imaging, and found prominent efferent projection of RGCs on Kölliker-Fuse (KF) nucleus in contralateral mesencephalon. This connectivity was confirmed by CTB retrograde labelling via intracerebroventricular (ICV) injection on KF nucleus. These sub-population of RGCs were identified as ON-OFF and direction selective ganglion cells by morphological and electrophysiological examinations. The function of this newly discovered circuitry is currently unknown yet, but is proposed to be related with the potential effect of visual inputs on body vital signs such as respiratory rhythm, heart rate or blood pressure, as KF nucleus directly project to medullary regions.

Disclosures: H. Lu: None. **L. Zhang:** None. **Z. zhao:** None. **M. tan:** None. **K. chen:** None. **K. So:** None. **C. Ren:** None.

Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Support: NIH R01 MH095878

NIH Fellowship F32MH108317

Title: Structural integrity of the geniculostriate pathway in schizophrenia and bipolar disorder

Authors: *E. A. REAVIS[1,4], J. LEE[1,4], J. K. WYNN[4,1], K. L. NARR[2], S. N. NJAU[3], A. C. MCNAIR[4], J. E. IGLESIAS[4], M. F. GREEN[1,4];

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Abstract: Patients with schizophrenia and related psychotic disorders show specific, persistent abnormalities in visual perception. Converging evidence suggests that some such perceptual deficits are linked to structural and functional abnormalities in brain areas near the bottom of the visual processing hierarchy, including the earliest stages of cortical visual processing (i.e., V1). However, it remains an important and unresolved question whether the structure and function of subcortical visual pathways are abnormal in such disorders. Early studies that used Diffusion Tensor Imaging (DTI) to assess the integrity of white-matter connections in schizophrenia reported reduced Fractional Anisotropy (FA) in the geniculostriate pathway (i.e., the optic radiations: the primary subcortical input to V1). These findings were interpreted as evidence of reduced structural integrity for the primary input pathway to the cortical visual system. However, DTI methods have advanced substantially since those early studies, and modern methods such as high-dimensional diffusion imaging and probabilistic tractography have not been used to assess the geniculostriate pathway in schizophrenia. Moreover, the structural integrity of the geniculostriate pathway has not been investigated in bipolar disorder, which emerging evidence suggests might manifest with perceptual abnormalities similar to those in schizophrenia. Therefore, we investigated the structural integrity of the geniculostriate pathway in a population of 32 patients with schizophrenia, 31 patients with bipolar disorder, and 30 matched controls. We used 64-direction DTI imaging and bidirectional probabilistic tractography between individualized thalamus and V1 seed regions to localize the geniculostriate pathway in each participant, and calculated FA within those tracts. Contrary to early reports, we found no evidence of diminished FA in either schizophrenia or bipolar disorder. This suggests that the structural integrity of the geniculostriate pathway is relatively intact in schizophrenia and bipolar disorder. Thus, perceptual deficits in the disorders are not likely attributable to degraded transmission of visual information to visual cortex via the geniculostriate pathway.

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Poster

529. Subcortical Visual Pathways

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 529.04/VV18

Topic: D.06. Vision

Title: The impact of chronic amphetamine treatment on the superior colliculus

Authors: ***A. TURNER**[1], P. G. OVERTON[2], I. KRAEV[1], A. STRAMEK[1], C. L. ROSTRON[1], M. G. STEWART[1], E. J. DOMMETT[3];
[1]Life, Hlth. and Chem. Sci., The Open Univ., Milton Keynes, United Kingdom; [2]Univ. of Sheffield, Sheffield, United Kingdom; [3]King's Col. London, London, United Kingdom

Abstract: Increased distractibility refers to a reduced ability to discriminate relevant from irrelevant information. It affects many people, as it is a symptom of a number of different conditions including attention deficit hyperactivity disorder, dyslexia, schizophrenia, and depression. It can also occur naturally as a person ages. The most effective form of treatment for increased distractibility are psychostimulant drugs, such as amphetamine. Converging evidence suggests that a potential neural correlate for distractibility is the superior colliculus (SC). Alterations to this structure and its connections to the prefrontal cortex have been found to disrupt distractibility. Furthermore, dose dependent decreases in visual activity within this area have been found after acute amphetamine administration, suggesting a possible therapeutic locus of action for the drug. However, these drugs are taken therapeutically for an extended period of time, limiting the conclusions that can be drawn from acute administration. As such we investigated the effects of chronic amphetamine on collicular function. Hooded lister rats were treated chronically with a range of doses of amphetamine (2 mg/kg, 10 mg/kg and 5mg/kg) or a vehicle, using a therapeutically-relevant oral dosing method. The effect of chronic treatment was then determined using behavioural, electrophysiological and anatomical measures. Collicular-dependent behaviour was measured using a visual distractibility task and height-dependent air-righting. In addition, to rule out confounding effects of altered locomotor activity, activity monitoring was conducted. Local field potential and multiunit activity were recorded from the superficial SC under urethane anaesthesia in response to a whole field light flash, in the presence of an acute amphetamine or saline challenge. Finally, anatomical measures included SC and whole brain volume calculations using the Cavalieri principle and cell counts for both neurons and glia. Results showed no significant impact of vehicle treatment both chronically and during acute electrophysiology. There were, however, a number of significant effects of amphetamine on behaviour and electrophysiological measures, indicating the SC may be a site of action for the distractibility-modulating effects of psychostimulants.

Disclosures: **A. Turner:** None. **P.G. Overton:** None. **I. Kraev:** None. **A. Stramek:** None. **C.L. Rostron:** None. **M.G. Stewart:** None. **E.J. Dommett:** None.

Poster

529. Subcortical Visual Pathways

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Program#/Poster#: 529.05/VV19

Topic: D.06. Vision

Support: European Research Council (268970)

Title: Melanopsin contributions to image forming vision in mice

Authors: *A. E. ALLEN, F. P. MARTIAL, R. J. LUCAS;
Univ. of Manchester, Manchester, United Kingdom

Abstract: Melanopsin, found in ipRGCs (intrinsically-photosensitive retinal ganglion cells), is necessary for the accurate encoding of ambient light for subconscious visual responses (e.g. entrainment of the circadian clock; control of pupil size). There is, however, an emerging role of melanopsin in perceptual vision that currently is poorly understood. Particularly lacking is a description of the spatial/temporal information conveyed by ipRGCs that arises exclusively from melanopsin's photon capture, and what functional contribution this makes towards image-forming vision.
To isolate the particular components of the visual world which melanopsin might encode, we modified a commercially-available projection system so that each R, G and B channel was instead a combination of five independently controlled wavelengths (λ_{max} 405, 455, 525, 561, 630nm). This allowed us to present temporally-controlled, patterned stimuli in spectra that provided contrast for selected mouse photopigments. In this way, we produced patterns visible only to melanopsin or only rods and cones, and recorded electrophysiological responses in the visual thalamus (dLGN) of anaesthetised mice. Spatial and temporal receptive fields (RFs) for stimuli visible to melanopsin could be recorded in ~20% of dLGN neurons. These RFs were spatially similar but temporally distinct from those of rods and cones. Melanopsin responses were uniquely slow and sustained, and the dLGN's representation of spatial patterns invisible to melanopsin decayed over several seconds of static view. Using a movie simulating the impact of head/eye movements while viewing a natural scene, we found that the representation of differences in local radiance was enhanced for stimuli visible to melanopsin during periods of relative fixation. Thus, although melanopsin signals are outside the range required for high spatiotemporal acuity vision, these data reveal they are particularly useful in encoding coarse spatial patterns in radiance over longer timescales.

Disclosures: **A.E. Allen:** None. **F.P. Martial:** None. **R.J. Lucas:** None.

Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Support: Wellcome Trust No 095669

Wellcome Trust No 095668

Marie Curie Fellowship No 623872

Title: Modulation of visual responses by behavioral factors in mouse superior colliculus

Authors: *S. SCHRÖDER, M. KRUMIN, K. D. HARRIS, M. CARANDINI;
Univ. Col. London, London, United Kingdom

Abstract: Cells in the superficial layers of mouse superior colliculus (SC) receive visual input from retinal ganglion cells and from primary visual cortex (V1). Previous work has shown that visual responses in V1 cells are modulated by non-visual factors such as intrinsic, ongoing activity and the animal's speed of locomotion. Does the same hold for cells of superficial SC? We used two-photon microscopy to record the activity of neuronal populations in the superficial SC of awake mice that were head-fixed and free to run on a floating ball. Our implant exposed the posterior SC, which is normally covered by a large sinus, while leaving the cortex intact. We used Gad-Cre mice to distinguish excitatory from inhibitory neurons, and expressed GCaMP6f via unconditional virus injection. We mapped receptive fields (RFs) with sparse noise stimuli and used drifting sinusoidal gratings to measure direction tuning. In addition to locomotion, we monitored eye position and pupil dilation.
We used a linear model to predict responses to gratings as a function of stimulus identity, locomotion speed, and ongoing activity, i.e. neural activity just before stimulus onset. A large number of neurons responded to the grating stimuli, and these were divided into two populations that were excited and suppressed by the stimulus. For the majority of neurons, visual responses were also influenced by behavioral factors. Both running speed and ongoing activity had diverse effects on visual responses, suppressing them in some cases and enhancing them in others. The effect distributions were similar for excitatory and inhibitory neurons. Direction tuning was affected by running speed and ongoing activity, but neither factor was correlated with a consistent increase or decrease in direction selectivity across the population of neurons. Neurons that were strongly tuned for direction, however, were less strongly modulated by running speed and ongoing activity than less strongly tuned neurons. Responses of more strongly tuned neurons were also on average more likely to be suppressed by high amplitudes of ongoing activity whereas less tuned neurons showed increased responses.

Our results show that neuronal responses in the superficial layers of superior colliculus are not solely driven by visual inputs but that for some neurons, non-visual factors such as ongoing activity and running speed are strong determinants of their responses. We plan to test whether these non-visual influences are inherited from cortex or even retina.

Disclosures: S. Schröder: None. **M. Krumin:** None. **K.D. Harris:** None. **M. Carandini:** None.

Poster

529. Subcortical Visual Pathways

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Program#/Poster#: 529.07/VV21

Topic: D.06. Vision

Title: The effect of electrode conditions for surprachoroidal-transretinal stimulation system on electrically-evoked potentials.

Authors: *K. NISHIDA[1], H. SAKAGUCHI[1], M. KAMEI[2], T. FUJIKADO[1], K. NISHIDA[1]; [1]Osaka Univ. Grad. Sch. of Med., Suita/Japan, Japan; [2]Aichi Med. Univ., Nagakute, Japan

Abstract: PURPOSE: We have developed a visual prosthesis system called the suprachoridal-transretinal stimulation (STS) system. The purpose of this study was to determine the effect of electrode conditions that would affect electrically-evoked potentials (EEPs) from the rabbit visual system. **METHODS**: A scleral pocket (3x5 mm) was created just over the visual streak in anesthetized pigmented rabbits (weight, 1.9-2.7 kg). The STS stimulating electrode system was implanted into the pocket. We varied the stimulating electrodes with different height (0.3 or 0.5 mm), the reference electrodes in the different location (in the vitreous cavity, on the sclera which is 180 degrees from the stimulating electrode, or on the sclera which is near the stimulating electrode), and the reference electrodes with different surface area. EEPs were then elicited by each type of electrode conditions. Three sessions were repeated for each group.
RESULTS: For the height of the stimulating electrode, the implicit times of the EEPs were 33.7±0.28 ms and 33.4±0.37 ms, respectively, and the amplitudes were 71.4±13.7 μV and 79.2±20.4 μV respectively. These differences were not significant (P=0.725; P=0.680). The surface area of the stimulating electrode nor the location of the reference electrodes had no significant effect on the implicit times and the amplitudes of the EEPs (P>0.05).
CONCLUSION: The height of the stimulating electrodes (0.3 or 0.5 mm) , the location of the reference electrodes, and surface area of the reference electrode do not significantly affect the implicit times and amplitudes of the EEPs elicited by the STS system.

Disclosures: **K. Nishida:** None. **H. Sakaguchi:** None. **M. Kamei:** None. **T. Fujikado:** None. **K. Nishida:** None.

Poster

529. Subcortical Visual Pathways

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Program#/Poster#: 529.08/VV22

Topic: D.06. Vision

Support: Polish Ministry of Science and Higher Education and National Science Center N N303 820640

Title: Oscillations in background and visually evoked activity of cat superior colliculus neurons

Authors: ***W. J. WALESZCZYK**, A. T. FOIK, A. GHAZARYAN;
The Nencki Inst., Warsaw, Poland

Abstract: Oscillations are ubiquitous features of neuronal activity in sensory systems and are considered as a substrate for integration of sensory information. Several studies have described oscillatory activity in the geniculate visual pathway, but little is known about this phenomenon in the extrageniculate visual pathway. Here, we describe oscillations in the background and visually evoked activity in the cat superior colliculus, a retinorecipient structure in the extrageniculate visual pathway. Extracellular single unit activity was recorded in superficial layers of the superior colliculus of anasthetized (isoflurane in O_2/N_2O) cats during periods with and without visual stimulation. Autocorrelation, FFT and renewal density analyses were used to detect and characterize oscillations in the neuronal activity. Our study indicates that oscillations are common in the background and stimulus evoked activity of the superior colliculus and they were observed in the activity of about half of the recorded cells. Oscillations in visually evoked activity could appear in two forms - stimulus phase-locked, and stimulus phase-independent oscillations. Stimulus phase-independent and background oscillatory frequencies were very similar, suggesting that stimulus phase-independent oscillations may be a form of enhanced "spontaneous" oscillations. Stimulus phase-locked oscillations were present in responses to visual moving and flashing stimuli. In contrast to stimulus phase-independent oscillations, strength of phase-locked oscillations was positively correlated with stimulus velocity and neuronal firing rate. Our results suggest that phase-independent oscillations in superficial layers of the superior colliculus may be generated by the same mechanism(s) that lie in the base of "spontaneous" oscillations, while phase-locked oscillations may result from interactions within the intra-collicular network and/or from a phase-reset of oscillations present in the background activity.

Disclosures: **W.J. Waleszczyk:** None. **A.T. Foik:** None. **A. Ghazaryan:** None.

Poster

529. Subcortical Visual Pathways

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 529.09/VV23

Topic: D.06. Vision

Support: NWO VIDI

Title: Functional modulation of primary visual cortex by superior colliculus

Authors: **M. AHMADLOU**, *J. A. HEIMEL;
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Abstract: Attentional modulation of surround suppression in visual cortex is well known. However the underlying circuit is not studied yet. Here, using electrophysiological recordings and optogenetics, we show that superior colliculus, which is a key area in attentional network, modulates a part of the surround suppression in visual cortex, although the cortical response to full screen is not significantly affected. Interestingly, reduction of the surround suppression of V1 is not the only effect of silencing superior colliculus, but we saw reduction of orientation/direction selectivity of V1 neurons as well. There are two thalamic pathways from superior colliculus to visual cortex, one through lateral posterior nucleus (LP) and the other one through dorsolateral geniculate nucleus (dLGN). Therefore, in order to find out the pathways of these functional modulations, pharmacologically we silenced LP, while the superior colliculus was optogenetically manipulated. Surprisingly, the modulation of V1 effect is not through LP, but it's through dLGN. This study shows a new vital role of superior colliculus as a functional modulator of visual cortex and the importance of the tecto-geniculate pathway in cortical tunings, which was not considered before.

Disclosures: **M. Ahmadlou:** None. **J.A. Heimel:** None.

Poster

529. Subcortical Visual Pathways

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Support: NEI-NIH R01-EY022157 (ADH)

NEI-NIH F32-EY025530 (TAS)

NINDS-NIH T32-NS007220

Title: Selective ablation of luminance-sensing RGCs reveals specificity and stringency of pretectal targeting mechanisms

Authors: *T. A. SEABROOK[1], N. ISHIKO[1], O. S. DHANDE[1], V. P. WOOLEY[2], P. L. NGUYEN[2], A. D. HUBERMAN[1,3];
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Abstract: How do neurons select their targets during development? Despite the functional importance of linking specific neuronal subtypes to their correct brain targets, the developmental mechanisms that ensure this process occurs accurately, remain poorly understood. Here we address this important question in the context of parallel visual circuits in the midbrain pretectum. The pretectum consists of a dense collection of brain nuclei that mediate different reflexive behaviors, including optokinetic reflexes essential for image stabilization and pupillary light reflexes (PLR). These targets receive input from distinct types of retinal ganglion cells (RGCs), the output neurons of the eye. Luminance-sensing RGCs are born relatively early in development and initially send their axons to several incorrect targets during development (Osterhout et al., *Cell Reports*, 2014). After this initial mistargeting, they remove their inappropriate axon projections and maintain the correct ones to the olivary pretectal nucleus (OPN) and posterior pretectal nucleus (mdPPN). By contrast, later arriving RGC axons such as those driving optokinetic reflexes, are more accurate in their targeting, bypassing OPN and mdPPN and targeting other nearby retinorecipient nuclei instead, such as the nucleus of the optic tract (NOT), which is involved in image stabilization. One idea is that competitive interactions between these functionally distinct RGC subsets are important for target selection in the pretectum. To test this, we used Cre-dependent deletion of Tbr2, a transcription factor important for RGC survival, to specifically eliminate the early-projecting cohort of luminance-sensing RGCs. We then asked whether other intact types of RGCs redirect their axonal inputs to the vacated targets. Remarkably, even when the OPN and mdPPN were rendered devoid of their normal retinal input, the axons of other RGC types continue to avoid these targets. This suggests

that axon-target matching is controlled by molecular matching mechanisms that do not tolerate cross-wiring. In other words, the process likely involves signals that direct specific axons to their targets *and* signals that actively restrict the wrong types of afferents from innervating those same targets. The relevance of these findings to visual development, function and regeneration will be discussed.

Disclosures: **T.A. Seabrook:** None. **N. Ishiko:** None. **O.S. Dhande:** None. **V.P. Wooley:** None. **P.L. Nguyen:** None. **A.D. Huberman:** None.

Poster

529. Subcortical Visual Pathways

Location: Halls B-H

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Program#/Poster#: 529.11/VV25

Topic: D.06. Vision

Title: Vision drives accurate approach behavior during prey capture in laboratory mice

Authors: *J. L. HOY, D. ZHEN, G. SMITHERS, C. NIELL;
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Abstract: The ability to genetically identify and manipulate neural circuits in the mouse is rapidly advancing our understanding of visual processing in the mammalian brain. Recent studies have focused on investigating neural circuit function in mice performing simple behaviors under highly controlled, yet unnatural, conditions. However, identifying ethologically relevant visual behaviors in the mouse will strengthen efforts to understand the neural circuit basis of visual behavior. Here, we show that C57BL/6J mice robustly pursue, capture and consume live insect prey. We quantified the dependence of this behavior on visual cues by selectively blocking visual or auditory sensory input. Visual input is required for accurate maintenance of bearing to within 30 degrees of the target during pursuit, and for keeping the target at close range until final capture. Vision also significantly reduced the latency to initiate approach towards the target. While mice were able to capture prey using auditory cues alone, the accuracy of targeting and success rate for capture were much lower than for vision alone. Importantly, because mice can perform this behavior using purely visual input, we were able to estimate the specific features, such as prey size and motion, that triggered visually guided approach. These data provided an estimate of the natural operating range of the mouse visual system. These studies demonstrate that laboratory mice are capable of exhibiting dynamic and accurate approach behaviors that rely on vision. Furthermore, the robustness of this behavior will facilitate future studies that seek to identify and observe the underlying neural circuitry.

Disclosures: **J.L. Hoy:** None. **D. Zhen:** None. **G. Smithers:** None. **C. Niell:** None.

Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Support: FONDECYT 1151432

Title: Bilateral projections of the parabigeminal nucleus to the superior colliculus in Octodon degus

Authors: A. DEICHLER, 7800024[1], D. CARRASCO[3], T. VEGA[4], J. MPODOZIS[1], *G. J. MARÍN[2,5];
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Abstract: The parabigeminal nucleus (Pbn) is the mammalian homologue to the ithsmic complex of other vertebrates. A recent study has shown that optogenetic stimulation of the Pbn induces freezing and escape responses in mice, a result thought to be caused by a direct projection from the Pbn to the central nucleus of the amygdala. However the isthmic complex, including the Pbn, is tightly connected to the Superior Colliculus (SC), which upon stimulation of its medial aspect also triggers fear and avoidance reactions. As this Pbn-SC connectivity is not well understood, we investigated whether the behavioral consequences of the Pbn stimulation could be related to the topology of its projection to the SC. To that end, we studied the cytoarchitectural, neurochemical and hodological properties of the Pbn in a diurnal rodent, the *Octodon degus*. We performed immunohistochemistry for ChAT, *in situ* hybridization for VGluT2 mRNA, and neural tracer injections in the SC and Pbn. In sagittal ChAT preparations the Pbn appears as an elongated structure, with two subdivisions: a rostral Pbn, formed by a dense cluster of densely stained, large cell bodies; and a caudal Pbn, populated by less-dense and smaller somas. After *in situ* hybridization we found that both divisions are positive for VGluT2 mRNA, indicating that the Pbn neurons may co-release both acetylcholine and glutamate. Our tracer injections indicated that the two divisions of the Pbn constitute hodological specializations: in the caudal Pbn, we found labeled axon terminals and cells only in the side ipsilateral to the injected colliculus; in the rostral division, axon terminals and cells appear exclusively ipsilateral and contralateral, respectively, to the injected SC. CTb injections in the Pbn revealed a bilateral projection to the SC. The ipsilateral projection covers the whole extent

of the superficial layers of the SC especially in the central zone. The contralateral projection seems to be more specialized, as it forms a dense mesh of terminals, restricted to the medial SC, throughout its longitudinal extension. This medial projection is located where the upper visual field is represented, which in degus and other rodents has a large binocular overlap. It is known that looming visual stimulation restricted to this zone induce escape responses in mice. Thus, our results may link the functional data obtained by the visual stimulation of the upper visual field and the physiological stimulation of the Pbn and the medial SC, by suggesting that the contralateral, presumably glutamatergic projection of the Pbn drives binocular responses in the medial SC which mediates fear and escape visual reactions.

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Poster

529. Subcortical Visual Pathways

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Title: Different origins of visual feature selectivity in two major subcortical structures in the mouse

Authors: ***J. BARCHINI**[1], H. ACARON LEDESMA[2], Y.-P. CHEN[1], D. KOREN[2], J. CANG[1], W. WEI[2];
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Abstract: Direction selectivity (DS) and orientation selectivity (OS) are fundamental features of visual system function. These features have been observed throughout the visual system of many animal species, in structures including cortex, the lateral geniculate nucleus (LGN) of the thalamus, the superior colliculus (SC), and the retina. The mechanisms that give rise to these features at the different levels of visual processing are still a matter of intensive investigation. For retinorecipient regions, feature selectivity might be simply inherited from the retina. Alternatively, a more complex convergence of untuned retinal input, coupled with local processing, can lead to the emergence of those features.In this study we aim to distinguish between these possibilities by genetically manipulating these features at the level of the retina,

and assessing the consequences downstream. Our manipulation consists of a conditional knockout (KO) of the vesicular GABA transporter (Vgat) in ChAT-positive cells. We have previously shown that the KO results in a reduction of GABA release from starburst amacrine cells in the retina, leading to reduced DS in one subtype of direction selective ganglion cells (DSGCs, Pei et al., 2015). Here, we have performed new experiments using 2-photon calcium imaging to determine the response selectivity of large populations of retinal ganglion cells. These experiments show that both DS, and surprisingly, OS are reduced in the ganglion cells of these KO mice. We next studied the response properties of the two major subcortical visual structures that receive direct retinal input, the SC and LGN, in these KO mice. We have recently shown that neurons in the most superficial lamina (top 50 um) of the mouse SC are highly selective for stimulus direction (Inayat, Barchini et al., 2015), establishing it as a useful model to study the mechanisms of DS. In vivo 2-photon calcium imaging from this lamina in the SC of the Vgat-KO mice revealed a substantial decline in DS, compared to littermate controls. This phenotype closely mirrors our findings in the retina, and directly implicates DSGCs as the source of direction selectivity in the SC. In contrast, single unit recordings revealed largely normal levels of DS and OS in the LGN of the KO, suggesting that these properties might emerge de novo in the LGN through local processing of untuned retinal inputs. Together, our studies indicate that different mechanisms are at play in setting up feature selectivity in the two visual pathways.

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Poster

529. Subcortical Visual Pathways

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Title: Basal forebrain provides direct projections to inhibitory neurons in mouse primary visual cortex, but not to the parvalbumin-positive inhibitory subtype

Authors: *G. A. LEAN[1], Y. LIU[2], D. C. LYON[2];
[1]UC Irvine, Irvine, CA; [2]Anat. and Neurobio., Univ. of California, Irvine, Irvine, CA

Abstract: Basal forebrain projections to the primary visual cortex (V1) have been implicated in receptive field tuning and attentional modulation by several studies. This projection is thought to be cholinergic due to the nature of the basal forebrain region. Furthermore, these inputs are thought to have more of an impact on local cortical inhibition as extensive anatomical research shows the majority of neurons with cholinergic receptors (ChR's) in V1 are GABAergic, despite these cells only representing 20% of the neural population. In higher visual mammals such as primates, the parvalbumin(PV)-positive subclass is the most abundant of the inhibitory neurons with ChR's, whereas in rodents the proportion of PV neurons with ChR's is greatly reduced (Disney & Reynolds, 2014). Basal forebrain projections to V1 neurons are primarily to layer 5, where they can make direct synaptic contact, but acetylcholine is delivered to neurons in other layers less directly via diffuse extra-synaptic modulation known as 'volume transmission'. In mouse, we previously confirmed that basal forebrain projections synapse directly onto GABAergic, but not non-GABAergic neurons in V1 using a modified rabies viral injection targeted via a helper virus containing a GAD1 promoter (Lean et al. SFN, 2015). Here we use a new helper virus containing a large fragment of the FUGU PV promoter. Compared to our approach using the GAD1 promoter, we find that neurons projecting to PV cells are primarily local in origin; short range cortical projections (see also our submission by Lyon et al. SFN, 2016). Specifically, there is a distinct lack of labeling in the basal forebrain. This result indicates that basal forebrain projections do not directly synapse onto V1 PV neurons, suggesting that other inhibitory neuron subtypes are the direct targets. This is also consistent with data noted above showing fewer PV inhibitory neurons express cholinergic receptors in rodents, suggesting less of an emphasis of cholinergic modulation of this subtype. Whether or not V1 PV neurons receive direct basal forebrain inputs in higher visual species remains to be determined.

Disclosures: **G.A. Lean:** None. **Y. Liu:** None. **D.C. Lyon:** None.

Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Support: NHMRC APP1042893

Title: Early-life lesions of the pulvinar alter visual cortical anatomy and behaviour

Authors: *I. CARRIL MUNDIÑANO, D. M. FOX, W. C. KWAN, J. A. BOURNE; Australian Regenerative Med. Inst., Monash Univ., Clayton, Australia

Abstract: Previous studies from our laboratory showed that the medial portion of the inferior pulvinar (PIm) receives direct retinal which it relays to the middle temporal (MT) area of the visual cortex, both of which are pruned during development. This pathway is believed to underpin the early maturation of area MT and the dorsal stream network. Furthermore, after early removal of primary visual cortex (V1), this disynaptic pathway remains unpruned, which could be the neural substrate for the better preservation of vision observed in infants than adults following V1 lesions.
To study the influence of the retino-pulvino-MT pathway on the organization and maturation of the visual cortex, 4 neonatal (14-21 days) marmoset monkeys (Callithrix jacchus) received unilateral PIm ablations (NMDA, 100nl, 0.12M infusions). The anatomical changes of cortical areas post-lesion were mapped by Diffusion Tensor Imaging (DTI) analysis 6, 9, 18 and 36 weeks post-lesion, which revealed significant alterations of diffusion scalars in dorsal stream associated areas and the posterior parietal association cortex. Neural tracing experiments demonstrated that thalamic input from PIm to area MT was completely abolished. Moreover, reduced connectivity between V1 and area MT was detected. Immunohistochemical analysis of the dorsal stream tissue showed axonal damage and altered the excitatory/inhibitory balance. Two of the animals were trained to perform visually guided motor tasks with both hands. Behaviour experiments showed a significantly worse performance with the hand contralateral to the lesion site. Data presented here demonstrate that thalamocortical input from PIm in early life is critical for the correct development and maturation of cortical connections of dorsal stream visual areas, hence altering visually-guided motor behaviours.

Disclosures: I. Carril Mundiñano: None. **D.M. Fox:** None. **W.C. Kwan:** None. **J.A. Bourne:** None.

Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Title: Visual discomfort symptoms and nearwork activity in latino populations

Authors: *D. A. DEL CID, T. GORJI, J. R. MIER, N. URENDA, S. A. DREW;
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Abstract: Previous studies have indicated ethnic differences in visual perception in children but have focused very little on college aged adults (Klienstein, 2003). Current research suggests that the prevalence of myopia to be at 16.8% among the Latino population (Tarczy-Hornoch, 2006).

Previously, we observed a high prevalence of visual discomfort symptoms reported in a diverse population of college students. This in itself may not be entirely surprising as symptoms including ocular fatigue, perceptual distortions, and headaches can result from nearwork tasks such as reading or viewing computer screens, tasks common to the college experience. Given current reports of differences in myopia prevalence among different ethnicities, we were interested to determine whether similar distinctions could be observed in the reports of visual discomfort symptoms. Methods: We administered a battery of questionnaires to 451 college students, including two validated surveys for assessing visual discomfort symptoms, the Convergence Insufficiency Symptoms Survey (CISS) and the Visual Discomfort Survey (VDS) (Conlon, 1999) and demographic questions. Results: We found that participants who identified as being Latino/a had a significantly different pattern of results than those who did not. These data suggest that the prevalence of visual discomfort in the college population may be confounded by ethnic differences.

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Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Support: National Science Foundation Graduate Research Fellowship

Title: Defining the neural circuits that provide emotional significance to what we see

Authors: *L. D. SALAY[1], N. ISHIKO[1], P. L. NGUYEN[2], E. YAGHOUB[2], A. D. HUBERMAN[1,3];
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Abstract: How do we assign emotional value to what we see? Very little is known about where and how emotional valence information interfaces with brain regions associated with visual processing. We discovered using activity-driven 'TRAPing' of neurons with the immediate early gene cFOS (Guenther et al., 2013) that specific nuclei of the midline thalamus are activated during visual fear in mice. Interestingly, subsets of these nuclei receive input from visual areas and project to both the amygdala and prefrontal cortex- regions of the brain involved in emotionality and decision-making, respectively. By manipulating the activity of these midline

thalamic nuclei with chemo- and opto-genetics, we observed they can cause marked alterations in the behavioral responses to fear-inducing visual stimuli. To further investigate the role of the midline thalamus in emotional state, we also recorded pupillary responses during their activation. This induced pupil dilation, an indication of increased arousal. Thus, the midline thalamus is ideally poised to integrate visual information with emotional-processing brain regions and thereby help select optimal behavioral decisions. The relevance of these findings to studies of normal and pathologic emotional processing will be discussed.

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Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Support: DFG GR988/18-2

Title: Integrity of central visual pathways in patients with macular degeneration

Authors: ***M. MALANIA**[1], J. KONRAD[2], H. JÄGLE[2], J. S. WERNER[3], M. W. GREENLEE[1]; [1]Univ. of Regensburg, Regensburg, Germany; [2]Univ. Eye Hosp., Regensburg, Germany; [3]Univ. of California, Davis, CA

Abstract: Macular degeneration (MD) affects the central retina and leads to gradual loss of foveal vision. Although, photoreceptors are primarily affected in MD, the retinal nerve fiber layer (RNFL) and central visual pathways may also be altered subsequent to photoreceptor degeneration. Previous studies have shown that the absence of afferent stimulation due to retinal lesions causes a reduction of gray matter in the lesion projection zone of V1 (Plank et al., 2011). Ogawa and colleagues (Ogawa et al., 2014) also demonstrated compromised integrity of white matter pathways in patients with Leber's hereditary optic neuropathy and cone-rod dystrophy. Here we investigate whether retinal damage caused by MD alters microstructural properties of visual pathways using diffusion-weighted MRI. Six MD patients and 6 healthy control subjects participated in the study. Patients had large binocular central scotomas and were free from other ocular or neurological diseases. Retinal images were obtained by optical coherence tomography (OCT). Scan analysis was performed with Spectralis software. Diffusion-weighted images were acquired by a Siemens 3T head-only scanner. In addition, high-resolution T1-weighted structural images were collected for each subject. Data were processed using Vistalab software

(https://github.com/vistalab/vistasoft/). Diffusion-based tensor modeling and probabilistic fiber tractography based on ConTrack were used to identify the optic radiations (OR). We sampled 100,000 candidate fibers and kept about 30% of these by applying the ConTrack scoring algorithm. Each tract was partitioned into 100 equally sized segments. To obtain a summary of the tract profile data was averaged over 80 values of each tract profile excluding the first and last 10 segments in order to avoid partial-volume effects. Fractional anisotropy (FA), axial and radial diffusivity values (AD, RD) were calculated along the OR tract. We observed a significant main effect of subject group with respect to mean FA ($p < 0.00001$). Patients showed a significant decrease of AD ($P<0.005$) while RD ($p<0.00001$) values were increased. Analysis of OCT images revealed RNFL thickness differences with significant thinning in the eyes of MD patients compared to those of controls (average thickness: 86±5 vs. 94±3 μm, $p<0.00005$). Our results indicate that the RNFL and the white matter of the visual pathways are significantly altered in MD patients. Damage to the photoreceptors in MD leads to atrophy of the ganglion cell axons and to corresponding changes in structural properties of central visual pathways.

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Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Support: Sharon Stewart Aniridia Research Trust

Title: Decreased white matter integrity in visual tracts in people with aniridia

Authors: *C. R. BURTON[1], D. J. SCHAEFFER[1], A. M. BOBILEV[1], J. E. PIERCE[2], A. L. RODRIGUE[2], C. E. KRAFFT[2], B. A. CLEMENTZ[3], J. D. LAUDERDALE[4], J. E. MCDOWELL[3];
[1]Neurosci., [2]Psychology, [3]Psychology, Neurosci., [4]Cell. Biology, Neurosci., Univ. of Georgia, Athens, GA

Abstract: Aniridia is a congenital panocular disorder resulting from mutations of the *PAX6* gene important in both eye and brain development and characterized chiefly by iris hypoplasia. In addition to ocular abnormalities, differences in global brain volume and functional brain connectivity have been reported in humans with aniridia. Examining possible abnormalities of global white matter structure may be critical to understanding structural and functional deficits of

aniridia; few studies, however, have examined white matter integrity in this population. The current study utilized diffusion-weighted imaging to assess neural white matter integrity in 11 people with aniridia and 11 healthy comparison subjects, matched for gender and age. A map of the local connectome was calculated to compare quantitative anisotropy (QA), an index of white matter structure, in all voxels of the brain, revealing subcomponents of white matter tracts displaying differences in QA between people with aniridia and healthy comparisons. This model-free analysis indicated that QA was lower for people with aniridia only in portions of visual tracts and posterior, associated pathways. These differences demonstrate that abnormalities in white matter structure exist in people with aniridia and may underlie functional differences in vision and brain connectivity.

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Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Support: NIH Grant EY09593

Title: Temporal processing of sensory information in visuomotor regions of the mouse tectum and thalamus

Authors: *U. M. CIFTCIOGLU[1], V. SURESH[1], A. S. GORIN[1], K. R. DING[1], F. T. SOMMER[2], J. A. HIRSCH[1];
[1]USC, Los Angeles, CA; [2]Univ. of California, Berkeley, Berkeley, CA

Abstract: The retina has two main targets in the mammalian brain, the lateral geniculate nucleus of the thalamus (LGN) and the superior colliculus (SC). The geniculate comprises two divisions, the dorsal part (dLGN) provides input to the visual cortex and the ventral region (vLGN) is connected with subcortical sensorimotor structures including the SC. Recently we began to compare coding strategies in the vLGN with those in the SC to understand how these retino-recipient structures might communicate. The experimental techniques we used are whole-cell, single unit, and local field potential recording from anesthetized mice during the presentation of visual stimuli. Work in other species (Brecht et al., J Nphys 2004; Stitt et al., J Nphys 2013; Sridharan et al., J Nphys 2011) had shown that neurons in the SC encode visual stimuli in two

ways, by changing firing rate and by entraining gamma oscillations. Previously, we had asked if the vLGN might do the same and found that, for about a third of the population, full field stimuli evoked an increase in spike rate as well as gamma oscillations that were synchronized across trials. Moreover, for these cells, natural-movie stimuli often evoked oscillations whose strength waxed and waned during particular image sequences. Here we asked how the SC and the vLGN respond to the same classes of stimuli, with a focus on the temporal aspect of response. We found that gamma oscillations in the vLGN were typically evoked by luminance increments, whereas those in the SC were preferentially elicited by luminance decrements (also see Stitt et al., J Nphys 2013)—looming stimuli were particularly effective. Thus, the two regions integrate complementary information via oscillatory activity. A second difference between the two areas is receptive field size which, on average, is smaller in the SC than in the vLGN. We wondered if neurons with smaller receptive fields might display commensurately greater cross-trial temporal precision. Hence, we analyzed spike trains evoked by natural movies for cells whose receptive fields we had mapped quantitatively. There was not a strict relationship between receptive field size and spike precision nor a rigid dichotomy between the SC and vLGN. Temporal precision could fluctuate considerably during the time course of a given movie. For example, a thalamic neuron with a large receptive field might fire as precisely as a tectal neuron that had a small receptive field during a particular movie segment and vice versa (although firing in the SC seemed more precise overall). Future studies in which simultaneous recordings from both structures are made should shed light on how spike patterns in the SC and vLGN influence each other.

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Poster

529. Subcortical Visual Pathways

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Support: NIH Grant EY012716

Title: Development of feed-forward and feedback connections between dLGN and TRN.

Authors: *P. W. CAMPBELL, W. GUIDO;
Anatom. Sci. & Neurobio., Univ. of Louisville, Louisville, KY

Abstract: The reciprocal connections between the dorsal lateral geniculate nucleus (dLGN) and the thalamic reticular nucleus (TRN) play an important role in regulating thalamocortical activity during different behavioral states such as attention, wakefulness, and sleep. Axon collaterals of thalamocortical neurons provide feedforward excitatory input onto GABAergic TRN neurons, which in turn convey feedback inhibition to dLGN relay neurons. Here we examined when and how these circuits arise during early postnatal life. To address this, we employed mouse transgenics to visualize when these inputs appear and optogenetics to assess when functional patterns of connectivity emerge. We used a GAD65 transgenic mouse to visualize GABAergic projections to dLGN. Reticulo-thalamic fibers begin to innervate dLGN at early postnatal ages so that by the end of the first postnatal week TRN projections form a dense and uniform network throughout the entire nucleus. We conducted in vitro whole cell recordings in acutely prepared thalamic slices in transgenic mice that express channelrhodopsin 2 (ChR2) in somatostatin (SST)-containing neurons of TRN. Blue light stimulation of SST containing fibers in dLGN evoked weak postsynaptic inhibitory activity in dLGN during the first postnatal week. Inhibitory responses matured by postnatal weeks 2-3, showing a progressive increase in strength, paired pulse depression, sustained levels of inhibition at high rates of stimulation, and a potent suppression of excitatory retinal activity evoked by optic tract stimulation. We used a CRH-Cre mouse crossed with an Ai9 reporter line to visualize dLGN thalamocortical axons and their collaterals in TRN. At birth, thalamocortical axons could be seen passing through TRN, with fibers and their collaterals expanding throughout the visual sector by the end of week 1. In vitro recordings of TRN neurons from CRH-Cre x ChR2 mice, showed that blue light stimulation of ChR2 containing dLGN axon collaterals could evoke weak excitatory postsynaptic responses during the postnatal week 1. By postnatal week 2-3, excitatory responses became stronger and were of sufficient strength to evoke Na^+ spikes as well as low threshold Ca^{2+} spikes and burst firing. Thus, the development of feedforward excitation from dLGN to TRN, and feedback inhibition from TRN to dLGN follow a similar time course, with adult-like patterns of functional connectivity emerging at about three weeks of age. At this time retinogeniculate and geniculocortical connections are well established, descending corticothalamic connections are maturing, and thalamocortical rhythms are beginning to take shape.

Disclosures: **P.W. Campbell:** None. **W. Guido:** None.

Poster

529. Subcortical Visual Pathways

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 529.22/WW10

Topic: D.06. Vision

Support: R01EY024173

Title: GABAergic circuit interactions within the mouse dorsal lateral geniculate nucleus

Authors: *S. P. MASTERSON, G. GOVINDAIAH, W. GUIDO, M. E. BICKFORD;
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Abstract: The activity of the dorsal lateral geniculate (dLGN) is influenced by a variety of circuits that use gamma amino butyric acid (GABA) as a neurotransmitter. Extrinsic sources of GABAergic input include the thalamic reticular nucleus (TRN) and pretectum. Intrinsic inhibition in the dLGN is mediated by GABAergic interneurons that release GABA via both dendritic terminals and axon terminals. In order to understand the various ways in which these intrinsic and extrinsic GABAergic circuits interact, we carried out ultrastructural and optogenetic analyses using transgenic mouse lines that express green fluorescent protein (GFP) in TRN cells (GAD65-GFP) or intrinsic interneurons and pretectum (GAD67-GFP), or mice that express cre-recombinase in all 3 sources (GAD2-cre). Viral vector injections into the TRN, dLGN or pretectum induced the expression of a channelrhodopsin variant (ChIEF) and blue light pulses were used to differentially activate these 3 sources of inhibition. Electrical stimulation of the optic tract was used to activate retinogeniculate inputs. We found that within the mouse dLGN: 1) The TRN specifically innervates and inhibits relay cells, and the strength of this inhibition is depressed during high-frequency stimulation. 2) The pretectum innervates and inhibits both relay cells and interneurons; during high frequency stimulation, pretectum inhibition of relay cells is depressed, while pretectum inhibition of interneurons is facilitated. 3) Interneurons inhibit both relay cells and interneurons; high frequency stimulation depresses both connections. 4) Interneurons are connected via axon terminals. 5) GABAergic connections can inhibit retinogeniculate transmission by inhibiting relay cells, or disinhibit retinogeniculate transmission by inhibiting the intrinsic interneurons. These results reveal a complex organization of inhibition whereby the strength of the retinogeniculate excitation of relay cells is contingent on the firing frequency and temporal order of inhibitory input.

Disclosures: S.P. Masterson: None. **G. Govindaiah:** None. **W. Guido:** None. **M.E. Bickford:** None.

Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Support: NIH Grant EY012716

Title: The absence of retinal input affects the targeting and morphological development of interneurons of dLGN

Authors: *N. CHARALAMBAKIS, G. GOVINDAIAH, P. W. CAMPBELL, W. GUIDO; Anatom. Sci. & Neurobio., Univ. of Louisville, Louisville, KY

Abstract: The dorsal lateral geniculate nucleus (dLGN) of the mouse is a model system to understand visual circuit development. While most studies focus on dLGN relay cells and their connections, little is known about the factors regulating the development of intrinsic interneurons. Here we examined whether the fate, migration, and dendritic maturation of interneurons relies on retinogeniculate axon innervation. To accomplish this, we took a loss of function approach and crossed GAD67-GFP mice, which express green fluorescent protein (GFP) in dLGN interneurons, with *math5* nulls, mutants that lack retinal ganglion cells and central projections. In *math5* nulls, the absence of retinal input led to 50% reduction in the number of interneurons in dLGN. The vLGN, where dLGN interneurons course through, also showed reduced numbers of interneurons. Within dLGN, interneurons were unevenly dispersed with many cells clustered in the upper tier even at late postnatal ages when migration of interneurons into dLGN is complete. Neighboring somatosensory nuclei (VPM, VPL and PO) which normally lack interneurons now contained several, suggesting that interneurons were not lost in dLGN but simply misrouted during migration. The absence of retinogeniculate axon innervation also disrupted the pattern of dendritic growth among developing interneurons. Normally, interneurons undergo a period of exuberant growth during postnatal weeks 2-3, followed by a period of moderate pruning in week 4. However, interneurons from *math5* nulls failed to follow this developmental pattern, and remained simple in structure from birth to adulthood. Confocal 3-D reconstructions of biocytin filled cells revealed that at all ages interneurons from *math5* nulls retained 2-3 primary dendrites similar to WT, but displayed a sparse dendritic architecture, with fewer branches and highly constricted dendritic fields. Normally interneurons display 12-14th order branching but in *math5* nulls, branching rarely exceeded 3-4th order. Moreover, in *math5* nulls, axons collaterals were not readily apparent and dendritic protrusions which are thought to reflect F2 profiles were largely absent, suggesting possible alterations in interneuron synaptic connectivity. These data support recent findings showing that retinal input directs the postnatal migration of interneurons into dLGN (Golding et al., 2014). Our results also suggest that retinal axons provide additional trophic cues to support the growth and elaboration interneuron dendrites.

Disclosures: N. Charalambakis: None. **G. Govindaiah:** None. **P.W. Campbell:** None. **W. Guido:** None.

Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Support: NIH Grant EY024173

Title: Ontogenetic investigation of thalamocortical circuits for active vision

Authors: ***N. ZHOU**, S. P. MASTERSON, J. K. DAMRON, W. GUIDO, M. E. BICKFORD; Anatom. Sci. & Neurobio., Univ. of Louisville, Louisville, KY

Abstract: To define the cortical circuits activated by the mouse pulvinar homologue (lateral posterior nucleus or LPN), we carried out ultrastructural and optogenetic analyses using wild type (WT) mice and a transgenic mouse line that expresses green fluorescent protein in cortical interneurons (GAD65-GFP). We found that the LPN projects to interconnected regions of V1, the lateral extrastriate cortex, striatum and amygdala. In all cortical regions, terminals originating from the LPN primarily contact dendritic spines. For optogenetic analyses of these synaptic connections, viral vector injections in the LPN of WT or GAD65-GFP mice induced the expression of a channelrhodopsin variant (ChIEF), and blue light pulses were used to activate thalamocortical terminals in cortical slices maintained *in vitro*. Additional injections of cholera toxin subunit B conjugated to Alexafluor 488 (CTB-488) in V1, superior colliculus, or striatum/amygdala were used to identify subtypes of cortical projection neurons and target them for recording. Biocytin was included in the recording pipettes to subsequently examine the morphology of the recorded cells. From a total of 432 neurons recorded in cortical layers IV and V, 64% responded to optogenetic activation of LPN terminals with short, fixed latency excitatory synaptic potentials/currents (EPSPs/EPSCs). These monosynaptic responses were abolished in the presence of 1μM tetrodotoxin (TTX), but could be elicited in the presence of TTX when paired with 1mM 4-aminopyridine. Responses were blocked by the addition of AMPA (CNQX, 8 μM) and NMDA (APV, 10μM) receptor antagonists. The morphology of a total of 147 responsive cells was recovered and categorized as pyramidal (69%), spiny stellate (21%), or other (10%). The majority (74%) of interneurons recorded in GAD65-GFP mice (n = 34) responded to activation of LPN terminals, and the addition of a GABAa receptor antagonist (SR95531, 20μM) enhanced responses of both interneurons and pyramidal cells (up to 4 fold). Recordings targeted to specific projection neuron subtypes within layer V (corticotectal, n = 31, corticocortical, n = 33, and corticostriatum/amygdala, n = 68) revealed that the LPN primarily targets corticostriatum/amygdala cells (76% responsive). The majority of corticocortical neurons were also responsive (55%), but corticotectal cells were rarely responsive (6%). These results indicate that the LPN can strongly influence the activity of the visual cortex, and suggest that it

may act as a hub to dynamically coordinate body movements with the perception of visual signals.

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Poster

529. Subcortical Visual Pathways

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Topic: D.06. Vision

Support: NIH Grant EY012716

Title: Postnatal development of cholinergic input to the thalamic reticular nucleus

Authors: ***G. SOKHADZE**, W. GUIDO;
Anatom. Sci. & Neurobio., Univ. of Louisville, Louisville, KY

Abstract: The thalamic reticular nucleus (TRN) is composed of inhibitory neurons that surround the thalamus and act to modulate the transfer of sensory information to and from the primary sensory thalamic nuclei and cortex. A major input to TRN originates from cholinergic neurons of the basal forebrain and brainstem. Together these inputs are involved in modulating TRN activity in a behavioral state-dependent context, such as during sleep, wakefulness, and attention. Although the presence of cholinergic elements in TRN has been well documented, it is not known how this input initially develops. Moreover, TRN is subdivided into distinct sensory sectors, yet it is not clear whether the pattern of cholinergic innervation develops uniformly or differently across sensory sectors. To address these questions we utilized the ChAT-Cre transgenic mouse, crossed to a reporter line (Ai9), which allowed for selective visualization of cholinergic input within the visual and somatosensory sectors of TRN. At birth, cholinergic neurons of the basal forebrain and brainstem exhibited robust expression of red fluorescent protein tdTomato. By P7, extensive cholinergic axon innervation was seen in the ventral somatosensory sector of TRN, but not in the dorsal visual region. Cholinergic innervation of the visual sector began at P9 and resembled the adult like pattern by P14. To assess the synaptic properties of cholinergic input to TRN during postnatal development, we performed whole-cell patch clamp recordings in acute thalamic slices in ChAT-ChR2 mice, which express channelrhodopsin-2 (ChR2) in cholinergic terminals. We recorded light-evoked synaptic responses in TRN neurons of mice aged P5-P21 while activating cholinergic terminals with 3 ms. pulses of blue light. In the first postnatal week, cholinergic responses were purely excitatory,

and mediated by nicotinic receptors. By the second postnatal week, blue light stimulation evoked biphasic excitation-inhibition (E-I) responses, with the inhibitory component mediated by muscarinic receptors. During the first two weeks, the prevalence of cholinergic responses and peak amplitudes of excitatory component were greater for neurons located in the somatosensory sector (23/27 cells, 85.2%, 4.32 mV), compared to the visual sector of TRN (7/17 cells, 41.2%, 1.51 mV). Thus, the ventro-dorsal pattern of cholinergic of structural innervation matched the emergence and maturation of cholinergic synaptic responses in TRN. These data further suggest that the timing of functional cholinergic innervation of visual TRN occurs well after the establishment of adult-like patterns of retino- and reticulo-geniculate connectivity.

Disclosures: G. Sokhadze: None. **W. Guido:** None.

Poster

530. Representation of Faces and Bodies

Location: Halls B-H

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Program#/Poster#: 530.01/WW14

Topic: D.06. Vision

Title: Hand position modulates visually-driven fMRI responses in premotor cortex

Authors: *J. DE JONG[1], B. KLEIN[2], A. KEIZER[2], C. DIJKERMAN[2], S. DUMOULIN[2]; [1]Dept. of Exptl. Psychology, [2]Utrecht Univ., Utrecht, Netherlands

Abstract: While moving through our environment, it is crucial to monitor the immediate surroundings of our body to avoid harmful contact. Earlier studies suggest that neural populations in premotor cortex are responsive to visual space surrounding individual body parts and employ a body-centered reference frame. Thus, whereas most visual receptive fields are linked to retinal coordinates, these receptive fields are anchored to the space surrounding certain body parts. Here, we aim to characterize these neural populations in humans by examining how hand position modulates fMRI responses to a strong visual stimulus. Healthy participants viewed a contrast-defined, full-field stationary checkerboard pattern while we acquired fMRI-responses elicited by the visual stimulus using ultra-high field (7T) functional MRI (TR = 2 seconds, 2 mm isotropic). Participants were instructed to position their hand at different locations surrounding the visual stimulus. We define several regions of interest (ROI) based on strong co-activation with the visual stimulus in several parts of the brain, including premotor cortex. We compare the BOLD-amplitude within these ROIs between different hand positions. We find strong co-activation with our stimulus throughout the brain. The fMRI response elicited by the visual stimulus is modulated by hand position in several visually responsive regions, including premotor cortex. In contrast, responses in early visual cortex, including primary visual cortex,

are not modulated by hand position. Our results demonstrate that visually-driven neural responses in premotor cortex are modulated by hand position. We speculate that these neural populations may have their receptive fields anchored to the hand. Moving the hand to different locations near the visual stimulus modulates the amount of overlap between these receptive fields and the visual stimulus. These results provide opportunities to study these premotor neurons in more detail, paving the way for methods such as population receptive field (pRF) mapping.

Disclosures: J. De Jong: None. **B. Klein:** None. **A. Keizer:** None. **C. Dijkerman:** None. **S. Dumoulin:** None.

Poster

530. Representation of Faces and Bodies

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Topic: D.06. Vision

Support: FWO

IUAP

PF

Title: Coding of viewpoint, posture and identity of bodies in the macaque midSTS body patch.

Authors: *S. KUMAR, R. VOGELS;
KU Leuven, Leuven, Belgium

Abstract: FMRI studies in primates show body-category selective activations in temporal cortex. Single unit studies in the midSTS body patch revealed greater activity to images containing bodies compared to faces and objects. However, these neurons showed a marked body image selectivity, responding only to some body images. The body image selectivity may reflect encoding of body identity, posture and viewpoint. Here we examined the selectivity of the fMRI-defined macaque midSTS body patch neurons for these three variables. We designed a novel computer-generated stimulus set (n= 120 stimuli) depicting monkeys that differ in anthropometric features (identity; fat, normal and thin), with 5 different postures (2 threat, 2 submissive and 1 neutral posture), rendered at 8 viewpoints (45 deg step; rotated around the vertical axis). The internal facial features of the head were minimized and kept constant across all stimuli. The images were presented for 200 ms each during passive fixation in 2 rhesus monkeys. Single unit recordings showed a marked selectivity for viewpoint and posture. We

employed linear SVM with cross-validation to decode viewpoint, posture and identity from the responses of 88 midSTS neurons. First we asked whether we could decode one variable (e.g. viewpoint) when varying the two other variables (e.g. posture and identity). Decoding of identity and posture invariant viewpoint (classification accuracy: 73%; 200 ms bin; chance level : 12.5%) and viewpoint and identity invariant posture (50%; chance level: 20%) was well above chance (permutation test with shuffled stimulus labels; $p < 0.005$) but viewpoint and posture invariant identity decoding (36%) was barely above chance level (33%; $p = 0.04$). However, it was possible to decode identity for particular view and posture combinations (accuracy ranging between 57 and 95%; chance = 33%). Second, we asked how tolerant the decoding of one variable is for changes of the other variables by training e.g. viewpoint decoding at one particular identity and posture and testing at the same and other combinations of the latter two variables. This analysis revealed little generalization of posture and identity decoding with viewpoint, in line with the viewpoint selectivity of midSTS body patch neurons. Posture and viewpoint decoding generalized well across identities. Analysis of the viewpoint-dependent errors in pose decoding suggested a representation of head orientation. Viewpoint decoding generalized across the threat postures but not across the others. These data suggest that the output of midSTS body patch neurons are useful to decode the posture and orientation of bodies, with a contribution of head orientation.

Disclosures: **S. Kumar:** None. **R. Vogels:** None.

Poster

530. Representation of Faces and Bodies

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Support: Simons foundation grant ID# 325023

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Title: macaque face patch AM codes linear axes rather than exemplars in the face space

Authors: *L. CHANG[1], D. TSAO[2];
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Abstract: One central question about sensory systems is how sensory stimuli are represented by sensory neurons. Different approaches have been employed to study this question in lower sensory areas like retina and V1, but have not been successfully applied to higher areas due to

the complexity of computation. The use of natural images like animal or human faces has been successful in characterizing basic properties of higher level visual neurons. However, the difficulty in parameterizing natural images precludes a detailed characterization of these higher level neurons. To explore the full geometry of higher level sensory neurons in the stimulus space, we recorded responses of cells in face patches ML/MF and AM to a large set of realistic faces generated using an "active appearance" model that allowed us to link each image to a point in a 50 dimensional face space. This model separates the shape of the face, determined by the positions of a set of landmarks, from the intensity variation independent of the shape, i.e., shape-free appearance features. Principle component analysis was performed to extract the top 25 shape and the top 25 shape-free appearance dimensions from 200 real faces in FEI face database. Facial images were randomly drawn from the 50-d face space. To quantify tuning to these 50 dimensions, responses of each neuron were used to calculate a "spike-triggered average" (STA) stimulus, i.e., the average stimulus that triggers the neuron to fire. We found that middle face patch neurons were better tuned to shape features, while AM neurons were better tuned to shape-free appearance features. Furthermore, we found face cells were tuned along the "STA" axis in a ramp-like manner, with extreme responses occurring at extreme feature values. To distinguish between a model measuring the "distance" to an exemplar face and a linear model measuring projection along an axis in the space, we inspected tuning of AM neurons along axes orthogonal to "STA". Interestingly, we found AM neurons show nearly no tuning along the orthogonal axes. Furthermore, responses of face cells could be fitted by linear combination of 50-d features rather well (R^2=58±14%), much higher than models using exemplars. Linear tuning of AM neurons in the face space seems to be inconsistent with previous studies advocating the use of nonlinear mixing in representing complex cognition tasks[1]. Using computational modeling, we found that a linear model is more efficient and robust to noise than a nonlinear "exemplar" model in a face identification task, if the dimensionality of face space is high enough (≥10). 1 Rigotti, M. *et al.* The importance of mixed selectivity in complex cognitive tasks. *Nature* **497**, 585-590 (2013).

Disclosures: **L. Chang:** None. **D. Tsao:** None.

Poster

530. Representation of Faces and Bodies

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Support: JST Presto

NEDO

Title: A hierarchical probabilistic model of natural face and non-face images explains selectivity and tuning properties of macaque face processing neurons

Authors: *H. HOSOYA[1], A. HYVÄRINEN[2];
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Abstract: Faces may be the socially most important visual stimuli for primates. Recent macaque studies in the inferotemporal (IT) cortex identified several clusters of neurons specific to face processing and revealed various tuning properties regarding facial features. In this study, to shed light into the theoretical principles underlying such facial coding, we investigate a probabilistic model of naturalistic face and non-face images. Specifically, we constructed a hierarchical neural network model that starts with a simple fixed Gabor-analysis stage (which crudely approximates computation from V1 to V4) and proceeds to a mixture of two sparse-coding submodels, a "face submodel" and an "object submodel". The two submodels were each trained with face or non-face object images, after which, as expected, the internal representations in the face submodel looked like facial parts while those in the object submodel looked like object parts. The model neurons in the face submodel exhibited significant selectivity to face images compared to non-face images. This was due to the property of the mixture model that one submodel explains away an input image that is likely to belong to its class so that the other submodel needs no activity. Such selectivity could not be obtained from a sparse coding model trained only with face images. To further clarify how close the face-selective units are to the actual face-selective neurons, we simulated an experiment conducted by Freiwald and Tsao (2009) on the middle patch of face processing in macaque IT cortex. To this end, we input cartoon faces to the model network, while varying 19 parameters controlling the geometric layout of facial features (e.g., face aspect ratio, inter-eye distance, mouth size). The model units most often showed monotonic tuning to such parameters, responding maximally to one extreme parameter value and minimally to the other extreme, which is similar to the experimental results. Moreover, the average number of tuned dimensions per unit was around 3 and the tuned dimensions were most often related to face outline and hair; these were also consistent with the macaque experiment. Taken together, our results indicate that the coding strategy of facial geometric layout employed by the middle patch of face processing in macaque IT may be closely related to the sparse coding principle.

Disclosures: H. Hosoya: None. **A. Hyvärinen:** None.

Poster

530. Representation of Faces and Bodies

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Title: Distinct tuning for scene content across macaque face patches revealed through single-unit and fMRI responses to social movies

Authors: *N. PERWEZ, B. E. RUSS, D. A. LEOPOLD;
Cognitive Neurophysiol. and Imaging, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: A growing body of work has shown naturalistic videos, together with free viewing, can be used to probe aspects of visual processing that cannot easily be studied using more conventional flashed static images or short dynamic clips. For example, in simply measuring the consistency in the responses of a neuron or cortical area to repeated viewing of complex dynamic scene, one can assess its involvement in processing the information contained therein. Here we applied such an inter-viewing method to investigate the extent to which regions of the macaque face processing system depend on the presence of faces and bodies. We designed two types of five-minute movies, one replete with primate social content and one containing dynamic scenes of storms and natural disasters, but devoid of animals. We showed these movies to seven macaques. In three animals, viewings were conducted during whole-brain fMRI scanning. In five animals (including one who participated in the fMRI study) neural activity was recorded in the middle lateral (ML), anterior fundus (AF), or anterior medial (AM) face patches using chronic microwire electrode bundles. The fMRI results demonstrate that the majority of the ventral visual stream, including the face patches, in the three animals responded consistently across viewings, regardless of whether animals were present in the videos. A bilateral region, that was overlapping the middle face patch, did not to show significant inter-viewing correlations during viewing of the non-social movies, indicating that activity in these areas is contingent on social stimuli. To better understand how neurons in this region and complementary face patches were responding to the movie content, we implanted microwire bundles in 3 of the face patches in the anterior temporal lobe. Similar to the results of the fMRI, single-unit responses differed across the three face patch regions. ML face patch neurons responded more consistently to the movies that contained animals, whereas AF and AM neurons responded with similar consistency to the two movie types. These preliminary results demonstrate a fundamental difference in the nature of selectivity in different fMRI-defined face patch, and illustrate the complementary information offered by free-viewing naturalistic paradigms in efforts to understand the neural mechanisms of high-level visual processing.

Disclosures: **N. Perwez:** None. **B.E. Russ:** None. **D.A. Leopold:** None.

Poster

530. Representation of Faces and Bodies

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Support: US National Institute of Neurological Disorders and Stroke (R01NS078396)

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Title: Intracranial recording of neuronal population responses to different categories of visual stimuli in the human ventral temporal cortex

Authors: *N. REN[1,2], S. SALEHI[2,3], J. PARVIZI[2];
[1]Dept. of Psychology, Peking Univ., Beijing, China; [2]Lab. of Behavioral & Cognitive Neurosci. (LBCN), Stanford Human Intracranial Cognitive Electrophysiology Program (SHICEP), Dept. of Neurol. & Neurolog. Sciences, Stanford Univ., Stanford, CA; [3]Shiraz Neurosci. Res. Center, Shiraz Univ. of Med. Sci., Shiraz, Iran, Islamic Republic of

Abstract: Despite a large number of neuroimaging work, it remains to be determined to what extent the power and time of onset of activity within the category-specific populations of neurons in the human ventral temporal cortex (VTC) are varied in response to non-preferred stimuli or different sub-categories of the preferred stimuli. We recorded intracranial ECoG signals from epilepsy patients implanted with subdural electrodes over their VTC while they viewed different categories of faces (e.g., human, mammal, and bird faces) and many other non-face categories. Here, we present a comprehensive map of the profile of neuronal population activities across many sub-regions of the human VTC in response to various categories and sub-categories of visual stimuli.

Disclosures: N. Ren: None. **S. Salehi:** None. **J. Parvizi:** None.

Poster

530. Representation of Faces and Bodies

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Title: From agents to actions to interactions: Uncovering multiple social networks in the primate brain

Authors: *J. SLIWA, W. A. FREIWALD;
The Rockefeller Univ., New York, NY

Abstract: Our brain continuously decodes the complex visual scenes unwinding in front of us: both the nature of material entities we perceive, such as objects and individuals, and their immaterial interactions. Interactions are recognize quickly and effortlessly by primates: They understand fights, grooming and plays, but also colliding objects that exchange forces following physical laws of classical mechanics. Interactions are fundamental in that they reveal hidden properties of objects, e.g. their weight or material, and of individuals, e.g. their dominance status or relationship, and by doing so they determine and teach the observer about its own position and prospects regarding those entities. However little is known about the brain regions that track and process social and physical interactions. In order to chart these regions, videos of three types of interactions 1) social interactions between monkeys, 2) interactions between monkeys and objects or their environment and 3) physical interactions between objects, were projected to four rhesus monkeys being scanned for fMRI acquisition with contrast agent. Whole-brain activity for watching blocks of interactions was compared to the activity for watching control videos of monkeys making no actions, objects moving with no interactions, landscapes and scrambled motion videos using Fixed Effects (FFX) Generalized Linear Model (GLM) group analysis and conjunction analyses. We show that watching interactions over-activates the STS, but engages also two sets of regions located outside: 1) it activates the fronto-parietal mirror neuron system (mapped independently using a classic localizer) more than watching non-interactive goal directed behaviors that define the system; 2) in the case of social interactions, it additionally exclusively activates the medial-prefrontal cortex (mPFC), a putative temporo-parietal junction homolog and the temporal pole (TP) that appear to correspond to the human mentalizing network. These two networks are fed differentially by patches of STS cortex (mapped independently using a classic Face-Object-Body patch localizer): face patches co-activate with the social brain, while body patches co-activate with both the mirror neuron system and the social brain. These results demonstrate that combining individuals or objects into evocative units

modulates basic mechanisms of object and individual perception in the STS, they reveal the mirror neuron system's nature of node of convergence between the social and non-social brain, and suggest that human unique and sophisticated mind-reading ability evolved from the faculty shared with our monkey kin to read social interactions.

Disclosures: J. Sliwa: None. **W.A. Freiwald:** None.

Poster

530. Representation of Faces and Bodies

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Grant-in-Aid for Scientific Research on Innovative Areas, "Sparse Modeling" from JSPS KAKENHI (26120529, 16H01555)

Title: Multiplex representation of information in face responsive neurons of monkey area TE

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Abstract: Neurons in the temporal visual cortex are known to be responsive to faces. We previously reported that face-responsive neurons in monkey area TE represent information about a global category, namely human faces vs. monkey faces, earlier than information about more detailed categories about the faces, i.e. facial expression and/or identity. To investigate how the information about the global category and that about the fine categories (facial identity and expression) were represented by each single neuron, we analyzed activities of 119 face-responsive neurons in area TE of two rhesus monkeys (*Macaca mulatta*), performing a fixation task. The test stimuli were colored pictures of monkey faces (4 models with 4 expressions) and human faces (3 models with 4 expressions). Each stimulus was presented for 400 ms. The number of spikes was counted in a 50-ms time window slid by 50 ms from 50 ms to 500 ms after the stimulus onset. To determine activity dependent on the global category, one-way ANOVA

(factor = human vs. monkey) was applied to the spike counts in each time window for each neuron. To determine activity dependent on the fine category in the human faces and that in the monkey faces, two-way ANOVAs (factors = identity and expression) were applied. Sixty-four of the 119 neurons (53%) showed significant dependence on both the global and fine categories. Fifty-three of the 64 (82%) showed significant dependence on the global category simultaneously or earlier than the effect of the fine category. The distribution of the timing of the earliest time window of the global category effect was significantly earlier than that of the fine category effect (Wilcoxon signed rank test, p=0.004), replicating the results of our previous studies. Eighty-five of the 119 neurons showed significant dependence on the fine category; 37 and 25 neurons showed dependence exclusively on the monkey faces and on the human faces, respectively, while the remaining 23 neurons showed dependence both on the monkey and human faces. Of the 37 neurons showing the fine category dependence only on the monkey faces, more than half the neurons (23/37, 62%) had dependence on the expression, but the dependence was frequently observed (13/23) together with the identity and/or interaction between them. Similarly, of the 25 neurons with the fine category dependence only on the human faces, most of them (22/25, 88%) showed dependence on the identity, but the dependence was frequently observed (12/22) together with the expression and/or interaction between them. These results suggest that different types of the fine information, even across different global category members, are multiplexed in single neurons.

Disclosures: **H. Ichikawa:** None. **Y. Igarashi:** None. **Y. Masutani:** None. **K. Kawano:** None. **M. Okada:** None. **Y. Sugase-Miyamoto:** None.

Poster

530. Representation of Faces and Bodies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 530.09/WW22

Topic: D.06. Vision

Title: Enhansive and suppressive face neurons form two distinct physiological networks for course and fine face representations in inferotemopral cortex

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Abstract: A network of clusters of face selective neurons in the inferior temporal (IT) cortex of monkeys has been found mostly by studying the excitatory responses to face stimuli. But the

spiking activities of a relatively large proportion of neurons in IT are suppressed following presentation of faces. Most of these neurons are located outside the face clusters. Contribution of these suppressive neurons in face representation is not clear. We recorded the spiking activities of single neurons in the inferior temporal cortex of two macaque monkeys while they passively viewed face and non-face images and compared the contribution of enhanced (ENH) and suppressed (SUP) face selective neurons in face representation. Fifty-six of 176 neurons selectively responded to faces by either increasing or decreasing their spiking activities compared to their baseline. The neural code for fine and coarse face information was largely different between the ENH and SUP face selective neurons at single cell and population levels. Compared to ENH neurons, SUP neurons showed higher (lower) sparseness values for highly similar individual faces (face category). Pattern of activity in the SUP neural population discriminated highly similar face images better than ENH face neural populations. But ENH neural population discriminated faces from non-face objects better than SUP neural population. Our data show that ENH and SUP neurons form two distinct physiological neural networks in IT cortex with widely different coding properties: a course-scale (ENH neurons) and a fine-scale (SUP neurons) system. These face processing functional subunits may index fine-to-course shape similarity in parallel depending on task at hand.

Disclosures: S. Salehi: None. **M. A.Dehaqani:** None. **H. Esteky:** None.

Poster

530. Representation of Faces and Bodies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 530.10/XX1

Topic: H.02. Human Cognition and Behavior

Support: ERC grant facessvep 284025

Title: A face-selective ventral occipito-temporal map of the human brain with intracerebral potentials

Authors: *J. JONAS[1,2], C. JACQUES[1], L. MAILLARD[2], B. ROSSION[1];
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Abstract: Human neuroimaging studies have identified a network of distinct face-selective regions in the ventral occipito-temporal cortex (VOTC) with a right dominance. To date, there is no evidence for this hemispheric and regional specialization with direct neural measures. To address this issue we recorded neurophysiological activity from 1678 contact electrodes

implanted in the VOTC of 28 epileptic patients. They were presented with natural images of objects at a rapid fixed rate (6 Hz), with faces interleaved as every 5th stimulus (1.2 Hz, Fig. 1A). High signal-to-noise ratio face-selective responses were objectively identified and quantified (at 1.2 Hz and harmonics) throughout the whole VOTC (Figure 1B). Face-selective responses were widely distributed across the whole VOTC (Fig. 1B-2A), but also spatially clustered in specific regions (Fig. 2B). Among these regions, the right lateral fusiform gyrus (latFG) showed the largest face-selective response by far (quantification analysis in Fig. 1C), offering the first supporting evidence of 2 decades of neuroimaging observations with direct measures. In addition, 3 distinct face-selective regions were disclosed in the anterior temporal lobe (Fig. 1B), a region that is undersampled in neuroimaging due to artefacts. Contacts responding only to faces were found in these regions, suggesting that they are involved in dedicated face processing functions. These observations provide a comprehensive mapping of visual category-selectivity in the human VOTC.

Figure 1

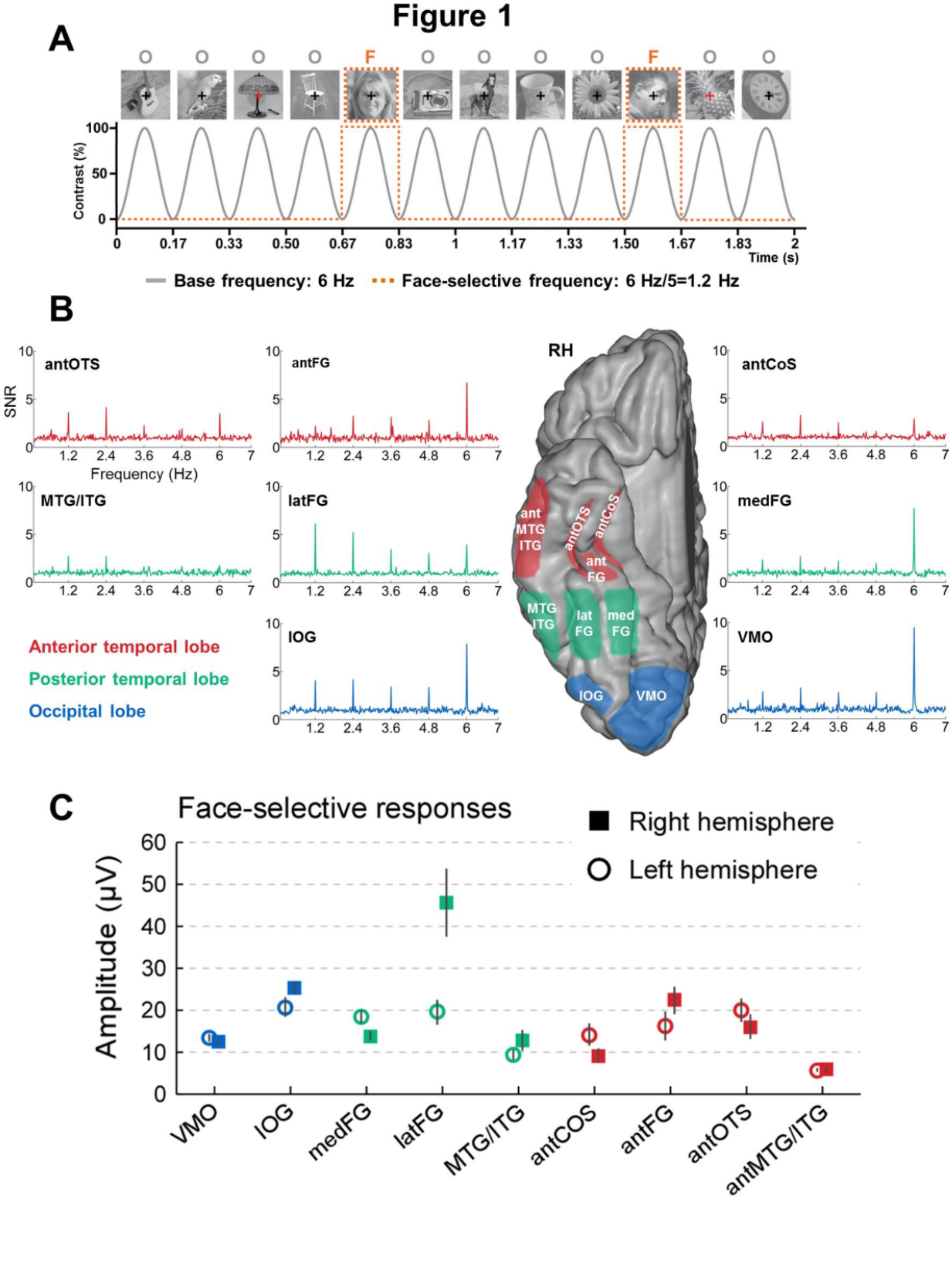
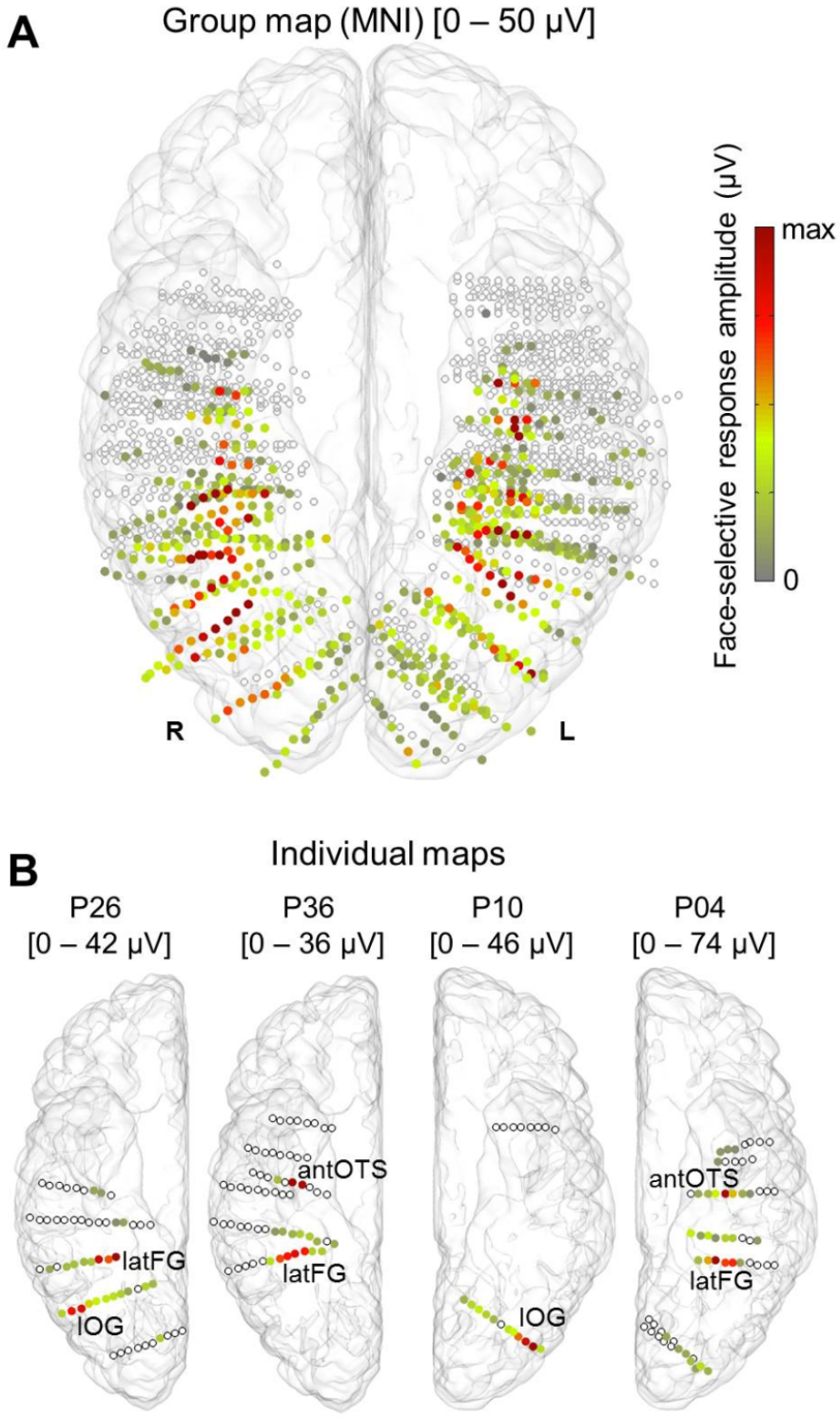

A
O O O O F O O O O F O O
Contrast (%)
100
50
0
0 0.17 0.33 0.50 0.67 0.83 1 1.17 1.33 1.50 1.67 1.83 2
Time (s)
Base frequency: 6 Hz
Face-selective frequency: 6 Hz/5=1.2 Hz
B
SNR
antOTS
antFG
antCoS
MTG/ITG
latFG
medFG
IOG
VMO
Frequency (Hz)
Anterior temporal lobe
Posterior temporal lobe
Occipital lobe
RH
ant MTG ITG
antOTS
antCoS
ant FG
MTG ITG
lat FG
med FG
IOG
VMO
C
Face-selective responses
Right hemisphere
Left hemisphere
Amplitude (μV)
VMO
IOG
medFG
latFG
MTG/ITG
antCOS
antFG
antOTS
antMTG/ITG

Figure 2



Disclosures: **J. Jonas:** None. **C. Jacques:** None. **L. Maillard:** None. **B. Rossion:** None.

Poster

530. Representation of Faces and Bodies

Location: Halls B-H

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Program#/Poster#: 530.11/XX2

Topic: H.02. Human Cognition and Behavior

Support: NIH R01 NS078396-01

Marie-Sklodowska Curie Actions individual fellowship (DecoMP_ECoG, 654038)

Title: How distributed is the face selective information in the temporal cortex: an ECoG study

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[1]Lab. of Behavioral and Cognitive Neurosci., Stanford Univ., Palo Alto, CA; [2]Dept. of Computer Sci., Univ. Col. London, London, United Kingdom; [3]Dept. of Psychology, Univ. of California, Berkeley, CA

Abstract: Introduction Previous fMRI studies have shown that face processing is distributed over the ventral temporal cortex (VTC). In Haxby et al., 2001, it is further suggested that after removing highly face selective areas (determined by a univariate analysis), combining the remaining signals allows significant discrimination between faces and non-face stimuli using a multivariate approach. In our current study, we performed a similar analysis using electrocorticographic (ECoG) signals recorded from grids and strips of electrodes covering the human VTC surface. **Material and Methods** ECoG data were obtained from 6 subjects with refractory epilepsy implanted with electrodes as part of their clinical assessment. Three patients had electrodes implanted on the left and three on the right hemisphere. Patients performed two experimentally controlled tasks involving the presentation of images of faces and of non-faces (places, limbs, objects, words, numbers, false fonts). The data from the first task was used to detect 'face-selective' VTC channels based on a locally multivariate analysis of the power of the signal epoched between 0 and 350ms after onset in the high frequency broadband (HFB, 70 to 177Hz). The data from the second task was then analyzed to discriminate between faces and non-face epochs based on (i) all VTC channels, (ii) all VTC channels except the 'face-selective' channels from task 1 after FDR correction, (iii) all VTC channels except the 'face-selective' channels from task 1 without FDR correction ($p<0.05$) and (iv) the 'face-selective' channel with the highest selectivity score. **Results** The number of channels identified as 'face-selective' in the first task varied from subject to subject: S1: 2 with FDR correction (5, without correction), S2: 4 (8), S3: 5 (11), S4: 0 (5), S5: 1 (4), S6: 11 (13). Discriminating between faces and non-faces epochs in the second task led to significant classification (balanced accuracies ranged from 68.76 to 96.67%) for all subjects (i). However, when discarding the 'face-selective' channels, the discrimination reached chance level accuracy for 4 out of 6 subjects (ii) and 5 out of 6 (iii). This decrease in performance cannot be attributed to a loss of multivariate power, as only one 'face-

selective' channel (iv) led to significant detection of face processing. Therefore, the considered ECoG data suggests that face information present in 'face-selective' channels is sufficient and necessary for the modeling of face processing in the VTC.

Disclosures: J.V. Schrouff: None. **V. Rangarajan:** None. **J. Parvizi:** None.

Poster

530. Representation of Faces and Bodies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 530.12/XX3

Topic: D.06. Vision

Support: NIH IRP

Title: Do rhesus macaques show the same visual preference for average faces as humans?

Authors: *O. TOMEO, L. G. UNGERLEIDER, N. LIU;
Lab. of Brain and Cognition, NIMH, Bethesda, MD

Abstract: In humans, facial attractiveness is a long-standing topic of active study in both neuroscience and social science, motivated by its positive social consequences. Non-human primates share similar social behaviors related to face processing and their underlying neural mechanisms with humans. For example, like humans, non-human primates can read information from conspecific faces about another's identity, mental state, and emotional state. However, it is unclear in non-human primates whether faces might also play a role in attraction as in humans and, if so, what kind of facial characteristics make a face preferred. To address these questions, we investigated the effect of averageness, a well-accepted factor that influences judgments of facial attractiveness in humans, on face preferences in monkeys. We tested three adult male rhesus macaques using a visual paired comparison task design in which they viewed pairs of faces (both individual faces, or one individual face and one average face). We found that monkeys looked longer at certain individual faces than other individual faces. However, unlike humans, monkeys did not prefer the average face over individual faces. Interestingly, looking times to individual faces reflected the norm-based face space, in which faces are encoded by their deviation from the average face: the more the individual face differed from the average face, the longer the monkeys looked at it. Taken together, our study provides new information about visual preferences for facial characteristics and behavioral evidence for the norm-based face space theory in monkeys.

Disclosures: O. Tomeo: None. **L.G. Ungerleider:** None. **N. Liu:** None.

Poster

530. Representation of Faces and Bodies

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 530.13/XX4

Topic: D.06. Vision

Support: EY10834

EY023268

P20GM103650

Title: Parallel effects of adaptation and distinctiveness on neural responses to faces

Authors: *O. S. GWINN, S. F. O'NEIL, M. A. WEBSTER;
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Abstract: Current models of face processing postulate a norm-based code in which individual faces are represented according to how they differ from an average face or norm. However, evidence for this model remains contested. We compared neural responses to an “average” vs. “distinctive” face and how these responses are biased by prior adaptation to the faces. An average female face was distorted by local expansion or contraction to create variations in distinctiveness while retaining common low-level image properties. Subjects passively viewed a rapid alternation between faces shown on a CRT monitor while electrophysiological responses were recorded with a 128-channel BIOSEMI system and analyzed in the frequency domain. In the first condition, 20 s sequences of faces alternated sinusoidally through contrast modulation between -50% contraction and +50% expansion at a rate of 6 Hz. In the second condition, the faces instead alternated between +100% expansion and the original undistorted face (0%). Responses to the +50/-50 alternation occurred only at the stimulation frequency of 6 Hz, indicating that responses to each distortion were equal. In contrast, the 0/100 alternation produced responses at both 3 Hz and 6 Hz, indicating different response amplitudes for the two faces, and consistent with a weaker response to the average face. Both conditions were repeated after 20 s adaptation to one of the face pairs. Adapting to either the +50% or -50% distortion produced a response at 3 Hz where before it was absent, consistent with a loss in sensitivity to the adapting distortion. Adapting to the 0% undistorted face increased the 3 Hz signal, such that the response to the average face was reduced. Adaptation to 100% distortion decreased the 3 Hz signal, such that the response to the average face was increased. This pattern is consistent with previous single-cell and fMRI studies suggesting weaker neural responses to average faces, and with EEG studies showing neural correlates of face adaptation. The present results further implicate a norm-based code by showing that variations in distinctiveness and adaptation have functionally similar effects on sensitivity (as measured by the evoked responses) to the faces.

Disclosures: **O.S. Gwinn:** None. **S.F. O'Neil:** None. **M.A. Webster:** None.

Poster

530. Representation of Faces and Bodies

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Program#/Poster#: 530.14/XX5

Topic: D.06. Vision

Support: EY-10834

P20 GM103650

Title: Asymmetric neural responses for expressions, anti-expressions, and neutral faces

Authors: ***C. MATERA**, O. GWINN, S. O'NEIL, M. WEBSTER;
Univ. of Nevada-Reno, Reno, NV

Abstract: Face recognition requires identifying both the invariant characteristics that distinguish one individual from another and the variations within the individual that correspond to emotional expressions. Both have been postulated to be represented with a norm-based code in which identity or expression are represented by the deviation from an average or neutral prototype. Different neural circuits have been characterized for different expressions, but it remains unclear how the encoding supporting the extraction of expression information maps onto the encoding supporting shape information and identity. We compared neural responses for expressions and anti-expressions, which are created by projecting an expression (e.g. a happy face) through the neutral face to form the opposite facial shape (anti-happy). The two faces thus differ from the norm by the same "physical" amount and thus have equivalent but opposite status with regard to their shape, but differ in their emotional salience. We asked whether neural responses to these complementary stimulus pairs were equivalent or asymmetric, and also tested for norm-based coding by comparing whether stronger responses are elicited by expressions than neutral faces. Stimuli were computer-generated facial images displaying the expressions 'happy', 'angry' or 'surprised' created using the program FaceGen, or photo-realistic images of actual individuals displaying the same expressions taken from the RADBOUD face database. Anti-expressions in the FaceGen images were controlled by inverting the sign of the expression magnitude, with the identity selected for an average male or female exemplar. Electrophysiological responses were measured for 7 observers with a 128 channel BIOSEMI system while observers passively viewed 30 sec of 6 Hz alternations of two facial images between each expression and its anti-expression or neutral face. Using a Fast Fourier Transform (FFT), recordings were analyzed in the frequency domain. A 3 Hz response was observed for all conditions except expression vs. neutral

for the FaceGen stimuli. This response was stronger in parietal-occipital regions and suggests an asymmetry between both expressions and anti-expressions and expressions vs. neutral (for photographic faces). An asymmetric response also occurred for male vs. female neutral faces but was stronger in occipital regions (possibly reflecting low-level image differences). Our results are consistent with a norm-based code for expressions but suggest stronger neural engagement for configurations denoting recognizable expressions.

Disclosures: **C. Matera:** None. **O. Gwinn:** None. **S. O'Neil:** None. **M. Webster:** None.

Poster

530. Representation of Faces and Bodies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 530.15/XX6

Topic: H.02. Human Cognition and Behavior

Title: How the human brain recognizes text-based emoticons.

Authors: *K.-W. KIM[1,2], B. JEONG[2], D. SHIN[2];
[1]Samsung Med. Ctr., Seoul, Korea, Republic of; [2]Grad. Sch. of Med. Sci. and Engin., Korean Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Introduction: An emoticon is a graphic of a facial expression that provides a sender's intention or emotion to a receiver in the absence of non-verbal communication such as body language and prosody. However, the question of how the human brain recognizes emoticons wasn't fully understood. Materials and Methods: Forty healthy volunteers (19 female age: mean ± s.d. =24.03 ± 3.28years) participated in the experiment. *fMRI and MEG experimental design:* Event-related design tasks consisted of 6 conditions: happy emoticons, happy faces, sademoticons, sad faces, scrambled emoticons, and neutral faces. *fMRI analysis:* First- and second-level analyses were performed with FSL'sFEAT. *MEG analysis:* Three distinct components were found in grand mean global field power (GFP) data corresponding to the M100, M170 and EPN to both text-based emoticons and face expressions. Then source analyses were performed on the three MEG components. Source reconstruction was implemented in the MATLAB package Brainstorm (Tadel et al., 2011). Results: *fMRI:* Two-way ANOVA was conducted with type of stimuli (emoticons, faces) and emotional valence (happy,sad, and neutral). Significant main effects of stimuli were found in the conjoined area, lateral occipital gyrus (LOC) and superior parietal lobule(SPL), the V1, the lingual gyrus, bilaterally, the left inferior temporal gyrus, the right ventromedial prefrontal cortex (VMPFC), and the right fusiform gyrus. Significant main effects of emotional valence were found in the inferior frontal gyrus (IFG), the paracingulate gyrus, bilaterally, the left orbitofrontal gyrus, and the left AIC.

MEG: Overall activity illustrated that the first source activity was estimated to the primary visual cortex at around 80ms (M100) after stimulus onset. This source disseminated anteriorly along the inferior occipito-temporal area, predominantly right side peaking at around 140ms (M170), and further onto the parietal regions at around 220ms (EPN). Time-courses extracted from source maps of the emoticons vs. faces differences also showed a dynamic pattern of activity.
Discussion: This study provided a text-based emoticon perception model. The input stimuli were processed first at around 80ms in the primary visual cortex as indexed by M100. Then face-specific appearances were processed in the FFA at around 150ms (M170) especially on the right.

Disclosures: **K. Kim:** None. **B. Jeong:** None. **D. Shin:** None.

Poster

530. Representation of Faces and Bodies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 530.16/XX7

Topic: D.06. Vision

Support: NARSAD Young Investigator Grant from the Brain and Behavior Research Foundation 21121

a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, Republic of Korea HI15C3175

Title: Task-dependent representations in visual cortex during face perception

Authors: *H. KIM, S.-H. LEE;
KAIST, Daejeon, Korea, Republic of

Abstract: When we see human faces, we can quickly and accurately recognize various information such as gender and race. Yet, despite the quick holistic processing in face perception, some aspects of the face are often emphasized whereas other aspects are not, depending on behavioral goal. Then, how is face information represented in the visual cortex when different aspects are emphasized? To address this question, we performed functional magnetic resonance imaging (fMRI) experiment, comprising two face tasks emphasizing either gender or race of the faces. In the gender task, participants were asked to decide whether the gender of the sample face image is the same with that of the test face image, whereas in the race task, they had to determine whether the sample face and the test face are from the same race category. We first found that there was no significant difference in behavioral performance

between the tasks. They showed comparable level of percent correct and reaction time for both tasks. Also we found that there was no difference in the BOLD response magnitude between the tasks. However, using multi-voxel pattern analyses, we found that anterior face-selective areas showed significantly discriminable patterns of response to individual faces during the gender task whereas the areas did not show the specific patterns during the race task. These results suggest that the distinctiveness of face representations in visual cortex is dependent on the nature of the task.
This work was supported by a NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation (grant number: 21121), and a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI15C3175).

Disclosures: H. Kim: None. **S. Lee:** None.

Poster

530. Representation of Faces and Bodies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 530.17/XX8

Topic: H.02. Human Cognition and Behavior

Title: Temporal dynamics of neural process relevant to intended percept of a face: An ERP study

Authors: *H. URATA, T. URAKAWA, K. MIZUNO, O. ARAKI;
Tokyo Univ. of Sci., Tokyo, Japan

Abstract: Face perception is indispensable for our lives. A number of previous EEG studies investigated early face-specific visual processing and reported an ERP component (N170) peaking at around 170 ms after the face image's onset. Since most of these studies employed experimental paradigms in which participants passively viewed face images, it remains largely unclear whether and how intention to perceive a face (a top-down process) would modulate early visual processing. We hypothesized that the top-down process for a face percept compared to that for other percepts would peculiarly affect the early visual processing, and tried to clarify this issue using morphed (ambiguous) face images. Seven healthy subjects participated in the present study. We made two morphed images using two unambiguous images (face and house). One of the morphed images was more perceived as a face (F) than a house (H), and the other was more perceived as H than F (these image were set based on a preceding behavioral experiment). The mean luminance was set identical for the two morphed images. There were two conditions: the intention to face (IF) condition in which participants were asked to perceive a face and the intention to house (IH) condition in which they were asked to perceived a house. In these

conditions, two morphed images were randomly presented. Results showed that the N170 was more enhanced in amplitude for the IF condition than for the IH condition. This response enhancement was comparable between F and H. As for a preceding VEP component P1, there was no such significant difference. These findings indicate that the top-down process for a face percept begins to appear at a latency of around 170 ms, and that the response enhancement in the IF condition compared to the IH condition reflects emergence of the top-down-mediated face-specific N170 component regardless of the difficulty to perceive a face. Furthermore, amplitude of the late positive component (LPC) under the IF condition was more augmented for H than for F at a latency of around 350-550 ms. In contrast, LPC amplitude under the IH condition was larger for F than for H. These findings imply that enhancement of LPC amplitude reflects the perceptual difficulty/conflict. The preset study suggests a temporal profile of top-down-mediated visual process of a face: the top-down process for a face percept would be implemented as early as around 170 ms regardless of the difficulty to perceive a face, and then the difficulty-dependent process would appear at around 350-550 ms.

Disclosures: **H. Urata:** None. **T. Urakawa:** None. **K. Mizuno:** None. **O. Araki:** None.

Poster

530. Representation of Faces and Bodies

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 530.18/XX9

Topic: D.06. Vision

Support: Supported by the NIMH IRP

Title: The ventral and dorsal visual pathways are functionally connected during configural face processing.

Authors: *V. ZACHARIOU[1], S. J. GOTTS[2], Z. N. SAFIULLAH[2], L. G. UNGERLEIDER[2]; [1]LBC:Section on Neurocircuitry, [2]LBC, NIH/NIMH, Bethesda, MD

Abstract: Configural face processing, the processing of the spatial relationships among the features of a face, is a vital component of face perception. If configural processing depends on spatial information, might this process involve interactions between the face-processing regions of the ventral stream and visuospatial processing regions of the dorsal stream? We explored this question in healthy adults by examining the pattern of functional connectivity between the right FFA as a seed (individually defined) and the rest of the brain in a same-different face detection task. Detection of configural relative to featural face differences led to significantly stronger functional connectivity between the right FFA and a-priori localized spatial processing regions of

the dorsal stream. In contrast, detection of featural relative to configural face differences led to stronger functional connectivity between the right FFA and other face-processing regions of the ventral stream, such as the right OFA and left FFA, as well as with the insula bilaterally. Further, these connectivity patterns correlated with reaction time performance: participants that responded slower on configural difference trials showed stronger functional connectivity between the right FFA, the left FFA and OFA and a-priori localized spatial processing regions of the dorsal stream, particularly within the left posterior parietal cortex. Conversely, participants that responded slower on featural difference trials showed stronger functional connectivity between the right FFA and anterior regions of the right inferior temporal gyrus, left insula and bilateral inferior frontal gyrus. Together, the findings suggest that the right FFA interacts with spatial processing regions of the dorsal stream during configural face processing and with face-processing regions of the ventral stream during featural face processing. Additionally, the extent of these interactions appears to depend on task demands.

Disclosures: V. Zachariou: None. **S.J. Gotts:** None. **Z.N. Safiullah:** None. **L.G. Ungerleider:** None.

Poster

530. Representation of Faces and Bodies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 530.19/XX10

Topic: D.06. Vision

Support: Grant by the Daimler and Benz Foundation

Title: How children and adults represent individual faces despite image variation - an fMRI adaptation study

Authors: *M. NORDT, K. SEMMELMANN, S. WEIGELT;
Dept. of Developmental Neuropsychology, Ruhr-University Bochum, Bochum, Germany

Abstract: Behavioral studies suggest that face recognition performance reaches its peak not until age 30. However, it is not yet fully understood which aspects of face recognition contribute to this prolonged development. A crucial facet of face recognition in daily life is the ability to recognize faces despite changes, such as varying viewpoints or changes in lighting. In the present study, we investigated the development of the individual-level selectivity of face-selective regions in children (aged 7-10 years) and young adults (aged 19-23 years) by using fMRI adaptation and by including multiple varying images of individual faces. Participants watched blocks of images while performing a simple detection task. There were three main conditions

including (1) the repeated presentation of a single image of one face, (2) the presentation of images of different faces and crucially, (3) the presentation of the same face in different images. In this third condition images contained slight variations of different factors such as lighting, viewpoint and facial expression. Preliminary analyses of the right FFA—defined based on adults' data of a functional localizer—revealed a main effect of condition with the highest signal in the different-faces condition, reduced signal for the same-face-different-images condition and lowest signal for the same-face-same-image condition, thereby indicating adaptation to same faces - even when presented in different images. Furthermore, results showed a main effect of group with higher signal for adults compared to children. Although numerically, the same-face-different-image-conditon was more strongly reduced for adults than for children, there was no significant group x condition interaction, indicating similar adaptation effects between groups and suggesting that individual-level selectivity for faces in the rFFA is similar in school-aged children and young adults.

Disclosures: **M. Nordt:** None. **K. Semmelmann:** None. **S. Weigelt:** None.

Poster

530. Representation of Faces and Bodies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 530.20/XX11

Topic: D.06. Vision

Support: ISF grant 296/15 to GA

Title: Behavioral and neural mechanisms underlying visual expertise in Congenital Prosopagnosia

Authors: ***N. WEISS**[1], G. AVIDAN[2];
[1]Psychology, Ben-Gurion Univ., Beer-Sheva, Israel; [2]Psychology, Ben Gurion Univ., Beer-Sheva, Israel

Abstract: Background: A major question in the face perception literature is whether faces comprise a distinct visual category that is processed by specialized cognitive and neural mechanisms, or whether face processing merely represents an extreme case of visual expertise. Methods: We address this issue by studying two women (O.H and S.I) with congenital prosopagnosia, a lifelong impairment in face perception in the absence of an obvious brain damage. Interestingly, despite their deficit, O.H reported having superior recognition skills for horses, and S.I reported excellent visual familiarity with flowers. These unusual circumstances in which visual expertise naturally emerges despite face perception impairment allow us to

disentangle neural mechanisms mediating visual expertise and in particular, to examine the extent of the modularity of core face related regions. We first conducted behavioural experiments to examine whether these objects of expertise are processed holistically, as previously claimed in the literature. We then employed fMRI to investigate the implications of expertise on neural responses to faces, horses and flowers. Results: Both CP experts employed similar behavioural and neural mechanisms for faces and objects of visual expertise. Specifically, they used local perceptual mechanism for processing faces and objects of expertise and exhibited similar enhanced fMRI BOLD response in right lateralized face-selective brain regions for faces and objects of expertise. Importantly, this pattern was dissociated from the response in control participants, experts and non-experts, in whom faces were processed holistically and elicited a greater response compared to horses/flowers within the same regions. Conclusions: These results suggest that visual expertise can be acquired despite impaired face perception mechanisms, and may be mediated by non holistic processing stratgeis and yet recruit right lateralized core face-selective regions.

Disclosures: **N. Weiss:** None. **G. Avidan:** None.

Poster

530. Representation of Faces and Bodies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 530.21/XX12

Topic: D.06. Vision

Support: NIH Grant EY023067

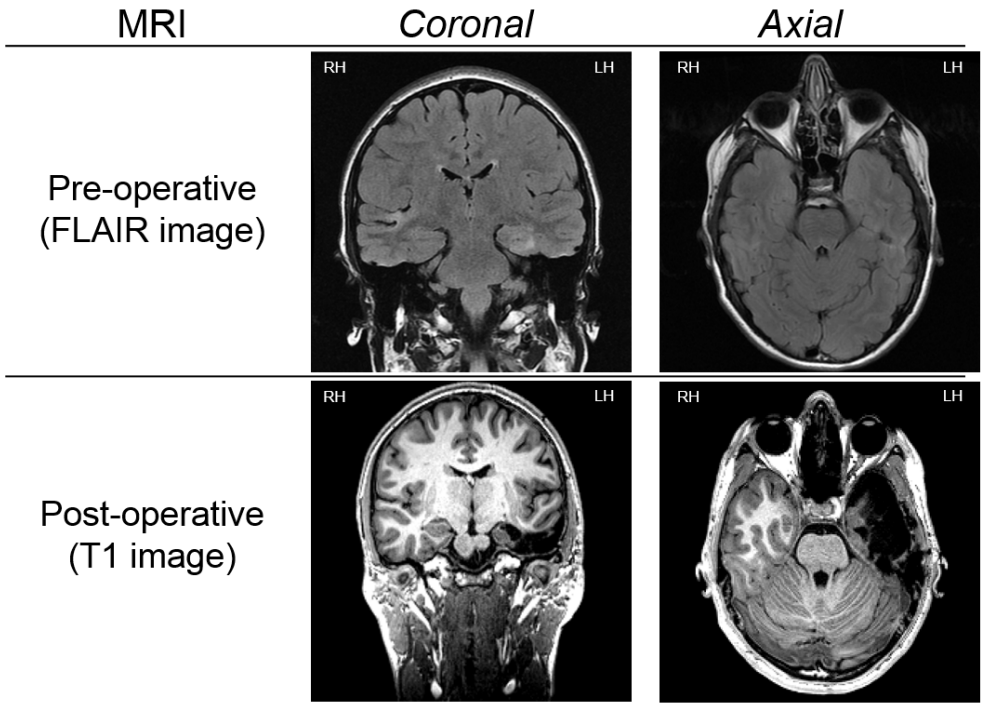
Title: Disruption of the visual system after temporal lobectomy

Authors: ***T. LIU**[1,2], A. NESTOR[3], K. KAY[4], M. VIDA[1,2], J. PYLES[1,2], X. ZHANG[5], C. PATTERSON[6], M. BEHRMANN[1,2];
[1]Psychology, Carnegie Mellon Univ., Pittsburgh, PA; [2]Ctr. for the Neural Basis of Cognition, Carnegie Mellon Univ. and the Univ. of Pittsburgh, Pittsburgh, PA; [3]Univ. of Toronto at Scarborough, Toronto, ON, Canada; [4]Univ. of Minnesota, Minneapolis, MN; [5]Natl. Inst. of Hlth., Bethesda, DC; [6]Pediatrics, Univ. of Pittsburgh, Pittsburgh, PA

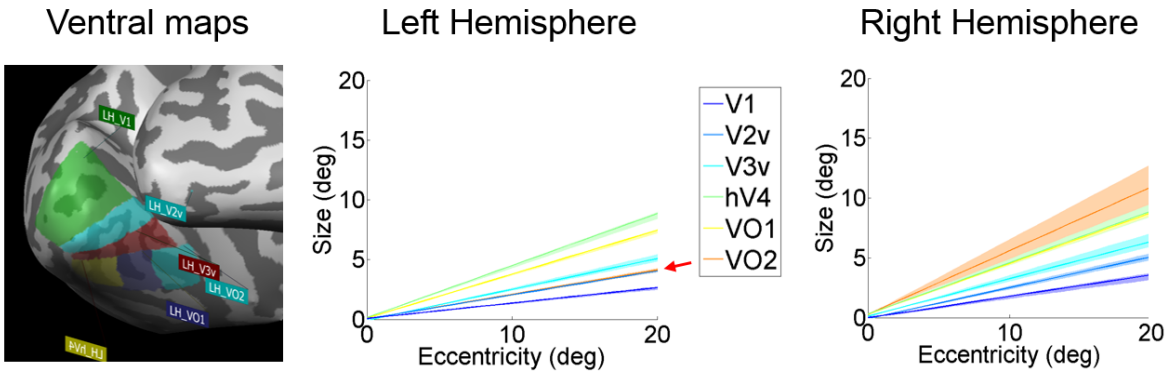
Abstract: Patterns of functional (re)organization in patients with hemispherectomy/lobectomy offer a unique opportunity to elucidate the nature and extent of cortical plasticity. First, in contrast with more common lesions, the extent of the damage in such patients can be extreme, yet, at the same time, very well controlled - both cortical and subcortical structures of the remaining hemisphere are typically intact. Second, the extent of the recovery is often

disproportionate relative to the extent of the damage - many compromised functions are regained partly or even completely. Here, we used fMRI to explore changes in the topography of cortical category selectivity and visual field maps (VFMs) in a 15-year-old, OT, who had a resection of the entire left anterior temporal lobe (ATL, see Figure 1A) due to medically intractable epilepsy. We also assessed the consequences of this resection for visual function using behavioral tasks targeting intermediate and high level vision. OT was impaired at face recognition (prosopagnosia) post-surgically but evinced equivalent IQ scores pre- and post-surgery in the high average range. fMRI scanning revealed normal topography and magnitude of selectivity in high-level visual cortex in response to common visual categories (face, places, object, and word). Also, imaging uncovered typical bilateral VFMs in early visual areas (V1-V3) of both hemispheres using a meridian mapping task. However, abnormalities in population receptive field (pRF) properties of the left hemisphere VO2 (Figure 1B) were observed using the pRF analysis. Further analyses address the mechanisms that potentially give rise to the abnormal VO2 properties, including the absence of top-down feedback from more anterior regions. Additional analysis and consideration of the prosopagnosia is also offered as well as functional connectivity measures within and between hemispheres. Figure 1. A) Pre- and post-surgical MRI. B) Ventral pRF maps in both hemispheres.

A. Pre- and post-operative MRI



B. Ventral pRF maps in both hemispheres



Disclosures: **T. Liu:** None. **A. Nestor:** None. **K. Kay:** None. **M. Vida:** None. **J. Pyles:** None. **X. Zhang:** None. **C. Patterson:** None. **M. Behrmann:** None.

Poster

531. Spatial and Feature-Based Attention

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PHS 5 R90 DA 23426-10

Title: Improved estimates of spike count covariance across populations of neurons

Authors: *G. VINCI[1], V. VENTURA[1], M. A. SMITH[2], R. E. KASS[1];
[1]Statistics, Carnegie Mellon Univ., Pittsburgh, PA; [2]Ophthalmology, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Neural recordings in large populations of simultaneously-recorded cells offer an opportunity to identify network interactions that vary across experimental conditions or behaviors. Statistical assessments of network interactions typically begin with a set of spike count variances and covariances (or correlations). For a small number of neurons these quantities, computed from data, have good statistical properties, but as the number increases a fundamental difficulty emerges: for N neurons there are on the order of N-squared variances and covariances, and as the dimensionality gets large it is hard to get good estimates of all them, together. Standard solutions to this problem involve some kind of "regularization," in which the variance-covariance matrix is stabilized somehow. A leading approach, known as Graphical Lasso, assumes sparse functional connectivity in the sense that the number of nonzero partial correlations—representing functional connections—is small; it effectively sets the smallest partial correlations to zero and "shrinks" the larger ones toward zero. This off-the-shelf method has two defects: first, functional connections may not be sparse; second, the Graphical Lasso takes no account of known neurophysiology. We introduce an alternative statistical procedure that takes advantage of a ubiquitous phenomenon: neurons that are close together tend to have correlated spiking activity. This new procedure identifies network structure by putting a soft constraint on correlations so that they tend to respect their known decreasing relationship with distance (and increase with tuning curve correlation), and it controls the false discovery rate. We have studied this new method and shown in numerical simulations that it can find true functional connections much more effectively than Graphical Lasso. We applied it to data recorded from

96-electrode Utah arrays in primary visual cortex (V1) of anesthetized macaque monkeys, where we had knowledge of the tuning curves and spatial arrangement of neurons. This work shows how knowledge of the biological factors that constrain correlated variability can be used to provide improved evidence of functional connectivity in populations of neurons.

Disclosures: G. Vinci: None. **V. Ventura:** None. **M.A. Smith:** None. **R.E. Kass:** None.

Poster

531. Spatial and Feature-Based Attention

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Pew Charitable Trusts

Title: Attention modulates specific subnetworks in mouse primary visual cortex

Authors: *A. M. WILSON, A. YAN, L. L. GLICKFELD;
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Abstract: Goal-directed attention allows for the prioritization of behaviorally-relevant sensory information and has been shown to improve both perceptual sensitivity and encoding of attended stimuli in sensory cortex. These attention-dependent changes in encoding differ according to the relationship of the neurons' tuning to the goals of the task, suggesting that perceptual changes are due to selective changes in the activity of functional subgroups of sensory cortical neurons. To determine if attention modulates specific subnetworks we monitored the activity of the diverse population of neurons in the primary visual cortex (V1) of mice performing a modality-specific, goal-directed attention task. In this task, head-fixed mice were cued on a trial-by-trial basis to switch between detecting changes in the orientation of a visual stimulus or the volume of an auditory stimulus; notably, visual stimuli before the change were symmetrical across trial types. We then tested the perceptual effect of attention by presenting rare, invalidly cued trials in which the mice were rewarded if they detected a change in the uncued modality. Mice had a lower probability of detecting invalidly cued targets, demonstrating that the cue is sufficient to set the expectation of, and attention toward, changes in the cued modality. Additionally, mice were less likely to respond to invalid targets on longer trials, indicating that they maintain, or even increase, their goal-specific attention as the trial progresses. In order to investigate the rapid neuronal modulation that occurs during task performance we monitored neuronal activity in V1

using two-photon imaging of GCaMP6. We analyzed two distinct epochs during task performance: the responses to the baseline stimuli (during anticipation of the stimulus change) and to the target stimulus. We found that, on visual trials the average responses to the baseline stimuli were transiently suppressed while responses to the target were enhanced. Interestingly, both of these effects were driven more by neurons tuned to the target stimuli than to the baseline stimulus. This suggests that attentional modulation in V1 is specific to neurons that provide the most information about the visual change. Investigation into the local and long-range circuits these neurons belong to will further elucidate the relationship of primary sensory processing to perception and behavior.

Disclosures: **A.M. Wilson:** None. **A. Yan:** None. **L.L. Glickfeld:** None.

Poster

531. Spatial and Feature-Based Attention

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Support: NIH EY063912

NIH MH063912

Gatsby Charitable Foundation

Title: Neural correlates of selective visual spatial attention in the mouse visual cortex

Authors: ***E. MCBRIDE**, S.-Y. LEE, E. CALLAWAY;
Salk Inst., La Jolla, CA

Abstract: Attention focuses our limited perceptual resources toward relevant stimuli in a distracter-filled environment. Work in primate visual cortex has shown that selectively attending to a location in the visual field enhances neural responses, signal to noise ratio, and perceptual sensitivity to stimuli presented there and suppresses responses and sensitivity to stimuli elsewhere. However, due to methodological limitations in primates, the specific neural mechanisms and circuits that influence attention remain mostly unknown. A more detailed description of these circuits requires simultaneous recordings from large populations of neurons, direct manipulation of the activity of specific neuron types, and observation of associated behavioral changes. Genetic tools in the mouse for selective manipulation of circuit components make these experiments possible.
When a mouse runs, visually-evoked neural responses are enhanced, similar to the effect of

attention. The circuit mediating this effect has been shown to involve cholinergic projections from the basal forebrain. This is likely a global alertness state primarily due to locomotion-induced processes. No study yet has examined selective spatial attention in mice, likely mediated by projections encoding a decision to attend. To investigate how neural circuitry is affected by attention and mediates changes in neural responses, we designed a mouse attention task that enables presentation of systematically varying stimuli, allowing generation of tuning curves from recorded neurons under changing attention conditions. Mice are shown identical drifting grating stimuli on two screens, one positioned in front of each eye. These stimuli can change in direction or spatial frequency from trial to trial. After a random stimulus duration, the mouse must detect a small change in contrast that could occur on either side and lick within a short time window to receive a water reward. The contrast change occurs more often on one particular side, and mice perform better on trials when the more likely change occurs, suggesting that they are selectively attending to that side. We are currently performing electrophysiological recordings using high-density laminar electrode arrays in visual cortices of these mice, and are able to record many units in both hemispheres with known laminar locations, measure receptive field properties, and compare visual responses under attended and unattended conditions. Results from these experiments will inform our choice of which neuronal populations to target with optogenetics in order to probe the role of those populations in the control of attention.

Disclosures: **E. McBride:** None. **S. Lee:** None. **E. Callaway:** None.

Poster

531. Spatial and Feature-Based Attention

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Support: National Eye Institute Intramural Research Program at the National Institutes of Health

Title: Detection-related activity in the primate superior colliculus supports action selection

Authors: *J. P. HERMAN, R. J. KRAUZLIS;
Lab. of Sensorimotor Res., NEI / NIH, Bethesda, MD

Abstract: The primate superior colliculus (SC) is known to be important for orienting. SC neuronal activity is related to overt orienting movements of the eyes and head, as well as to covert orienting of attention in the absence of eye movements. Here we provide evidence that SC's role is not limited to orienting but supports action selection in general by indicating the time and location of behaviorally relevant events. We recorded SC neuronal activity in two

rhesus macaques (n=80, 58) during a challenging covert attention task. The monkeys had to detect near-threshold changes in the saturation of two peripheral dynamic color "checkerboard" stimulus patches while maintaining central fixation. They were rewarded for reporting changes of the cued stimulus by releasing a joystick, and for ignoring changes of the spatially opposed foil stimulus by keeping the joystick held down. In addition to replicating cue-related modulation during the delay period, we found that difficult-to-detect changes in saturation caused vigorous phasic increases in activity in the majority of recorded units (76/80 and 56/58, respectively). The size of this detection-related activity was comparable to that found at stimulus or saccade onset but occurred in the absence of previously shown orienting effects (i.e., no saccades and after the shift in attention). When aligned on the stimulus change, we found that individual unit detection time (DT, the time at which increased activity became significant) followed shortly after the change (100-150ms). However, dividing activity into 3 quantiles by reaction time (RT) revealed a greater covariation between DT and RT when activity was aligned on the change than when aligned on the joystick-release; this indicates that the timing of detection-related activity is not determined by the stimulus change, but rather marks the animal's detection of the event. Furthermore, by comparing activity for hits and misses, we found mean choice-probabilities of 0.61 and 0.59 in the two animals, values greater than those reported in some sensory areas. Thus, in addition to its importance for orienting, neuronal activity in the primate SC appears to play a more general role in action selection by reporting when and where behaviorally relevant events occur.

Disclosures: J.P. Herman: None. **R.J. Krauzlis:** None.

Poster

531. Spatial and Feature-Based Attention

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T32 GM8244-23

T32 HD007151

University of Minnesota MnDRIVE Initiative

R01-EY014989

P30-NS057091

P30-NS076408

Title: Flavoprotein-based functional optical imaging reveals the distribution of attentional modulations across the surface of primary visual cortex

Authors: *S. G. WARREN, G. M. GHOSE;
Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: In many visual attention tasks, a small population of neurons in the brain is well-matched to the attended stimulus. Potentially, attending to a small region of space might therefore involve the selective targeting of attention modulation to those neurons whose receptive fields include the attended area. Because electrophysiological techniques measure relatively small numbers of neurons, such techniques are poorly suited to measuring the distribution of attentional modulation across a population. Conversely, because of the relatively poor spatial resolution of fMRI, it is also unable to address this question. Thus the fundamental issue of the precision of attentional targeting remains unresolved.
To address this, we collected functional optical images of flavoprotein autofluorescence from the primary visual cortex (V1) of two macaques while they performed an attention task involving a 3-by-3 array of small Gabors. Optical imaging provides a high resolution (<20 micron), spatially unbiased measurement of activity across V1, allowing us to discretely measure the pattern of V1 activity evoked by each individual stimulus under different attention conditions. We find that attention indeed enhances the evoked autofluorescence from the centers of the V1 regions stimulated by each stimulus. Consistent with electrophysiological studies in V1, these attention effects are modest (approximately 0.1% signal change) when compared to the strength of the evoked visual response (approximately 1%).
Crucially, while we observe sharply focal visually evoked responses, we found that the corresponding attentional modulations were spatially broad: the surrounding regions of V1 also exhibited attentional effects of similar magnitude. Moreover, when the subjects were cued that they should selectively attend to only a subset of the stimulus elements, we found that the pattern of attentional modulations over V1 remained crude and only grossly matched the spatial cue given to the animals. We propose that, within V1, attention is fundamentally imprecise and modulates both the center and surround and that this may explain why electrophysiologic studies of V1 often fail to observe the strong effects of attention that are suggested by fMRI.

Disclosures: S.G. Warren: None. **G.M. Ghose:** None.

Poster

531. Spatial and Feature-Based Attention

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Support: NIH EY018683

Title: Attention modulates LFP power and phase in primate V1 and LGN

Authors: *V. L. MOCK[1], J. HEMBROOK-SHORT[2], F. BRIGGS[2];
[1]Physiol. and Neurobio., Dartmouth Col. Geisel Sch. of Med., Lebanon, NH; [2]Geisel Sch. of Med. at Dartmouth, Lebanon, NH

Abstract: Increasing evidence suggests that attention modulates the activity of specific neuronal populations within a brain area and influences how information is communicated from one neuronal population to another. Parallel lines of research suggest that different neuronal populations, separated into laminar compartments, synchronize their activity at specific frequencies measured in the local field potential (LFP). We are examining how attention alters specific frequency modulations in LFPs recorded across the layers of primary visual cortex (V1) and frequency coupling between the visual thalamus (lateral geniculate nucleus or LGN) and V1. We record LFPs through electrodes placed within retinotopically-aligned regions of the LGN and V1 in awake-behaving monkeys performing a contrast or orientation change detection task that requires covert shifts in visual spatial attention. Our results show significant differences in power across the LGN and superficial, middle, and deep layers of V1 in both the cue and visual stimulus time periods of the task. However, we do not observe differences in LFP across attention conditions in the LGN or in the V1 layers. Interestingly, we do see a shift in the phase of LFP signals relative to the visual stimulus with attention in both V1 and the LGN. First, the relationship between differential phase and frequency is linear in the beta and low gamma bands, particularly between frequencies of 20-50Hz, suggesting a fixed temporal shift in LFP signals relative to the visual stimulus. Second, the slope of the linear phase-frequency curve is negative, indicating that LFPs on attend toward trials lead LFPs on attend away trials relative to the visual stimulus. Third, amplitude cross-correlation of V1 LFPs recorded on attend toward versus attend away trials shows a lead of approximately 10ms with attention, consistent with the fixed temporal lead predicted by the phase-frequency slope analysis. Furthermore, the attention-mediated phase lead is correlated with animals' behavior as phase leads are most significant on trials in which animals complete more difficult discriminations. Together, these phase shift results suggests that attention changes the timing of neuronal population activity relative to the visual stimulus with attention, possibly indicating that attention may be able to prime LGN and V1 neurons to respond more quickly to a given visual stimulus. These results support the notion

that attention selectively modulates the activity of specific neuronal populations within a cortical area in a behaviorally relevant manner and that this specificity influences how visual information is communicated in the early visual pathways.

Disclosures: **V.L. Mock:** None. **J. Hembrook-Short:** None. **F. Briggs:** None.

Poster

531. Spatial and Feature-Based Attention

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Program#/Poster#: 531.07/XX19

Topic: D.06. Vision

Support: NIH Grant F32 EY023165

NIH Grant EY018683

Title: Attentional modulation of V1 neurons differs across physiological cell types in the primate

Authors: *J. R. HEMBROOK-SHORT, V. L. MOCK, F. BRIGGS;
Physiol. and Neurobio., Geisel Sch. of Med. at Dartmouth, Lebanon, NH

Abstract: Attentional modulation of firing rate varies substantially across visual cortical neurons. For example, spatial attention directed to neuronal receptive fields can cause increases or decreases in firing rate. Whether variations in attentional modulation of neuronal firing rate depend upon physiological characteristics or laminar locations of neurons is not known. In order to explore relationships between visual physiology or laminar location and attentional modulation across visual cortical neurons, we measured attentional modulation and visual response properties of neurons spanning the cortical layers of primary visual cortex (V1) in awake-behaving monkeys. Animals performed a contrast-change detection task, which required covert shifts in visual spatial attention toward or away from gratings overlapping recorded neuronal receptive fields, and attentional modulation of firing rate was assessed by comparing spike counts across attention conditions. Visual physiology of recorded neurons was determined based on neuronal responses to drifting sinusoidal gratings varying in contrast, orientation, size, and temporal and spatial frequency. We observed significantly more attentional modulation of V1 complex cells compared to V1 simple cells ($p=0.00017$). Differences in attentional modulation of firing rate were also observed across cortical layers: complex cells in V1 granular and infragranular layers were significantly more modulated by attention compared to simple cells in V1 supergranular and infragranular layers ($p=0.0046$). We further observed relationships between visual physiology and attentional modulation across the V1 population and among

neuronal sub-populations within specific layers. Interestingly, we observed a significant relationship between contrast sensitivity and attentional modulation across all recorded V1 neurons (p=0.04), consistent with the notion that attention more strongly modulates neurons tuned to the stimulus features important for the task. We also observed relationships between feature tuning and attentional modulation among specific laminar sub-populations. Together these results suggest that attentional modulation depends upon visual physiology and varies across neuronal sub-populations in different layers.

Disclosures: J.R. Hembrook-Short: None. **V.L. Mock:** None. **F. Briggs:** None.

Poster

531. Spatial and Feature-Based Attention

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Program#/Poster#: 531.08/XX20

Topic: D.06. Vision

Support: DFG WE 5469/2-1

Title: Latency of stimulus onset and change transients in visual areas V1 and MT relate to reaction time

Authors: *B. SCHLEDDE, D. WEGENER;
Brain Res. Inst. / Univ. of Bremen, Bremen, Germany

Abstract: The ability to detect and respond to a relevant change in visual environment relies on the effective processing of visual information already in early visual areas. Selective visual attention actively supports the cortical processing of task-relevant information, allowing for facilitated behavioral performance, and local populations of neurons already in early visual areas are thought to play a critical role in enabling fast visual discrimination. In fact, the latency of rapid firing rate changes in response to abrupt change events was recently shown to be actively modulated by attention and to correlate closely with reaction time (RT). We here address the question of the underlying time course. Particularly, we ask whether response latencies to behaviorally relevant speed changes depend on the configuration of the network just before the change, or whether they indicate a more general characteristic of attentional modulation that persists throughout the trial. Furthermore, we ask whether attention-dependent latency shifts emerge within area MT, or alternatively, are inherited from a presynaptic population in primary visual cortex. We recorded from single neurons and local neuronal populations in visual areas MT and V1 while a macaque monkey performed a speed-change detection task requiring to detect an instant 100% speed increase at a spatially pre-cued moving Gabor patch. We found that

latencies and peak amplitudes of transient responses to abrupt changes in the visual stimulation differ substantially in trials resulting in fast or slow RTs. In area MT, this included the latencies of both early stimulus and motion onset transients as well as the speed-change transient occurring later in the trial. Interestingly, latency shifts in area MT were about twice as large as in area V1. Our results show that not only the transient response to the behaviorally relevant event, but also preceding changes to the target stimulus relate to RT, suggesting a persistent attentional impact throughout the trial. Furthermore, the fact that latency shifts from fast to slow trials in area MT are larger than latency shifts in V1 argues for an accumulation of latency advances along the processing pathway.

Disclosures: **B. Schledde:** None. **D. Wegener:** None.

Poster

531. Spatial and Feature-Based Attention

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DFG Grant FOR-1847

Title: Feature-based enhancement precedes object-based attentional modulation of MT responses in a delayed match-to-sample task

Authors: ***P. SCHWEDHELM**[1,2], S. TREUE[1,2,3];
[1]German Primate Ctr., Goettingen, Germany; [2]Bernstein Ctr. for Computat. Neurosci., Goettingen, Germany; [3]Fac. of Biol. and Psychology, Goettingen Univ., Goettingen, Germany

Abstract: A rapid and accurate identification of behaviorally relevant stimuli is often mediated by the deployment of attention. If target stimuli requiring a behavioral response are identified based on their stimulus features (e.g. their color or motion direction), feature-based attention enhancing searched-for features constitutes an efficient mechanism to enhance the neuronal representation of targets and reduce the one of distractors.
We measured feature-based enhancements of neurons in motion-selective area MT of two macaque monkeys while they performed a delayed match-to-sample task. Stimuli were identified as targets either based on their motion component, their color, or a conjunction of motion and color.
The responses to target stimuli matching the searched-for motion direction were enhanced by

attention, but we also found significant modulations of firing rates when animals did not attend to the motion but the color of the stimuli. Here, the time-courses of the attentional modulation in area MT revealed latency differences between feature-based modulations; with attention to the primarily encoded feature occurring earlier in time. This finding is consistent with a sequential activation of cortical modules and supports the notion of a unified, object-based attention system. However, when monkeys identified target stimuli based on a unique conjunction of color and motion, the observed modulation was not the sum of color- and motion-based matching tasks. Instead, we observed the biggest attentional effects for "partial targets" - distractor stimuli matching only one of the two searched-for features - consistent with a role of area MT in signaling potential targets, rather than targets. This finding suggests that feature-based attentional modulation represents an intermediate step preceding object-based attentional enhancement and will guide future research of attentional control circuits.

Disclosures: P. Schwedhelm: None. **S. Treue:** None.

Poster

531. Spatial and Feature-Based Attention

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NSF BCS143221

NEI R01EY026924

Title: Maintenance of spatial information modulates the gain and reliability of neuronal responses in areas V4 and MT

Authors: *Y. MERRIKHI[1], M. PARSA[2], B. NOUDOOST[3];
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Abstract: Holding a location in working memory has been shown to produce perceptual benefits at that location. Our lab has found that prefrontal cortex sends a persistent signal indicating the content of spatial working memory to extrastriate visual cortex during the delay period of a spatial memory task. Delay-period firing rates in extrastriate visual cortex, however, do not reflect the location held in memory, suggesting that any effect of this input from prefrontal

cortex must be sub-threshold. Such sub-threshold input could nevertheless render extrastriate neurons more sensitive to incoming visual signals. To test this hypothesis, we recorded the neural activity of areas V4/MT in rhesus monkeys while they performed a memory-guided saccade task. In this task, the monkey fixated for 1 second (baseline period) to start the trial. Then the target was presented for 1 second (visual period) and the monkey remembered its location for another 1 second (delay period). The fixation point then disappeared, and the monkey moved his eyes to the remembered cue location to receive a reward. To test whether the location held in memory alters the gain of visual responses, we presented brief (200 ms) visual probes in a 7x7 grid of locations near the one of cue locations, during both the baseline and the delay period. We compared the probe-evoked visual responses for trials with different target locations to see if the peak magnitude of the response changed based on the contents of spatial working memory. Remembering a location near the probe increased the peak response of single and multi units in areas V4/MT. Next, we used trials in which no visual probes were presented to examine the effect of spatial memory on the response reliability of single neurons in extrastriate cortex. The variability of neural responses in extrastriate cortex decreased when remembering a location near the neuron's RF. We hypothesize that these changes in reliability and sensitivity to visual signals are the result of sub-threshold modulation by the persistent spatial signal from prefrontal cortex. These effects of memory maintenance on the neural activity in extrastriate cortex, which are similar to the effect of covert spatial attention, could contribute to the behavioral benefits seen at a location held in memory.

Disclosures: **Y. Merrikhi:** None. **M. Parsa:** None. **B. Noudoost:** None.

Poster

531. Spatial and Feature-Based Attention

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Title: Moment-to-moment fluctuations of attention in macaque area V4

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Abstract: Spatial attention allows for selective processing of information in a specific area of the visual environment. Experimenters typically assess attention by comparing behavioral and neural responses between conditions with different attentional cues. However, with this approach, we lose information about meaningful trial-to-trial and moment-to-moment variability in attention. Recently, population neuronal recordings have enabled reliable estimation of spatial attention on individual trials (Cohen & Maunsell, 2010). We ask how the population level signatures of attention fluctuate from moment to moment in a trial. We recorded V4 population activity in macaques performing a cued spatial attention task in which they need to detect a gradual speed change in one of two drifting gratings (the target). On some trials, there was no speed change (catch trials). Considering catch trials in which the subject correctly withheld a response, we decoded the location of the attentional cue, and thereby the subjects' attentional state, using a linear classifier (support vector machine) from moment to moment. Classification accuracy peaked at 80% and had a time course that matched the statistics of target onset in the task. In other words, even though no targets were presented on catch trials, attentional fluctuations matched expected target saliency from validly cued trials. Furthermore, a classifier trained on population activity directly preceding stimulus onset also had 80% accuracy, but performed poorly when tested on neural activity from times after stimulus onset. This suggests that the representation of attention is capable of shifting dynamically from moment to moment, allowing maintenance of attention during adaptation to a changing visual field.

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Poster

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Title: Decoding V4 laminar population response during covert and overt attention

Authors: *W. W. PETTINE[1,2], N. STEINMETZ[3], T. MOORE[1,2];
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Abstract: The cerebral cortex's functional architecture is characterized by distinct lamination patterns, defined in part by cell type, connectivity and response properties. Previous studies of attentional modulation of V4 neural activity have found laminar differences in local field potentials, but not in spiking activity. We recorded spiking activity across the cortical layers of single cortical columns of area V4 in two rhesus macaques using a linear array microelectrodes during a selective attention task. For this task, the monkey fixated on a central point, while four gabor patches appeared, one in each quadrant. A cue then was presented indicating the gabor patch that was most likely to change orientation. If the orientation changed after a delay, the monkey was rewarded for saccading to the opposite gabor patch. The two gabor patches orthogonal to attended locations were considered controls. This task dissociated overt attention (the preparation and execution of orienting eye movements), from covert attention (selective processing without overt orienting). We then used machine learning classification to distinguish between behavioral conditions (covert, overt and control) on a trial-by-trial basis from population activity. Feature vectors consisted of neuronal ensemble firing rates and inter-spike interval distributions. Alternative pseudo-populations were also created through multi-ensemble feature grafting, which permitted independent testing of waveform classes, as well as the location of neurons within superficial or deep layers. Classification was applied to data during the epoch following the presentation of a cue that indicated which of four stimuli was the target of covert (or overt) attention. We found that neuronal ensembles and pseudo-populations of V4 neurons were able to distinguish between conditions in which overt, covert or no attention was directed toward the receptive field stimulus. The effect was significant for both narrow-spiking (putative interneurons) and broad-spiking (putative pyramidal) neurons, as well as for both superficial and deep layer neurons. These results suggest that the source of attentional modulation in V4 operates equally across cortical layers. Furthermore, our methods also provide a novel way to preserve raw population spike dynamics on a trial-by-trial basis when analyzing neural ensembles.

Disclosures: W.W. Pettine: None. **N. Steinmetz:** None. **T. Moore:** None.

Poster

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Title: The impact of "nuisance" variability on neural task performance

Authors: *N. ROTH[1], N. C. RUST[1,2];
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Abstract: Task performance is determined not only by the amount of task-relevant "signal" present in our brains, but also by the presence of "noise". While the relationship between signal and noise has been extensively described under conditions in which noise is limited to "trial variability", the impact of the noise introduced by task-irrelevant "nuisance" parameters is much less well understood. Generally, when noise is limited to trial variability, neural task performance (quantified by the measure d'[2]) grows with time; this relationship serves as a benchmark for many models of task performance. In contrast, we have determined that the presence of nuisance variability causes task performance to saturate at a time dependent not only on the amount of nuisance variation present but also on the overall firing rate. This result suggests that there may be a point beyond which neurons continue to fire but considering those spikes will leads to no net gain in information. To investigate the effect of nuisance variation on neural task performance in the brain, we analyzed neural responses recorded in inferotemporal cortex (IT) as macaque monkeys performed an "invariant delayed match to sample task" that required them to identify when a target object appeared in the presence of considerable nuisance variation. We found that neural task performance did in fact saturate at a time consistent with the monkeys' reaction times, and that a large component of this saturation could be attributed to nuisance variability. However, we also found that as a consequence of relatively low firing rates in our population, the amount of saturation was blunted relative to a high firing rate regime.

Disclosures: N. Roth: None. **N.C. Rust:** None.

Poster

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Support: NIH Grant EY014924

Title: Effects of parietal inactivation on saliency-driven eye movements

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Abstract: Salient regions in visual scenes draw attention and predict the direction of scanning eye movements, as well as modulate the activity of neurons within a number of structures which thought to be involved in visual attention. In particular, neurons within prefrontal and parietal cortex have been reported to represent stimulus saliency. We recently found evidence that the representation of saliency within prefrontal cortex, specifically within the frontal eye field (FEF) depends on the activity within parietal cortex. Following parietal inactivation, the ability of FEF neurons to signal the presence of a unique (popout) stimulus within an array of others was strongly reduced, while there was no change in their responses to single visual stimuli. In this study, we test whether this apparent reduction in saliency signals within the FEF is reflected by a reduction in the saliency driven visually guided eye movement within visual images. To inactivate parietal cortex, we implanted two stainless steel cryoloops within the intraparietal sulcus of a macaque monkey. Chilled methanol was pumped through the loops to induce localized hypothermia in the surrounding cortical tissue, thus silencing nearby neuronal activity. Simultaneously, we recorded the eye movements of the monkey while it freely viewed a large number (>1000) of static, complex images (eg. landscapes, cityscapes, fractals.) For each image we quantified the average saliency of the fixated regions using known saliency algorithms (Harel, Koch, & Perona, 2006). Our results thus far indicate that the average saliency of fixated regions for scanning eye movements directed into the intact hemifield is unchanged during parietal inactivation. However, the average saliency of fixated regions by eye movements directed into the inactivated hemifield is significantly reduced following parietal inactivation.

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Poster

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Title: Shedding light on attentional control: a combined opto-fMRI-electrophysiology study in monkeys

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[1]Neuro-And Psychophysiology, KULeuven, Leuven, Belgium; [2]Lab. for Neurobio. and Gene Therapy Dept. of Neurosciences KULeuven, Leuven, Belgium; [3]Neuro- And Psychophysiology, KULeuven, Leuven, Belgium; [4]Athinoula A. Martinos Ctr. for Biomed. Imaging, MGH, Charlestown, MA; [5]Radiology, Harvard Med. Sch., Cambridge, MA

Abstract: We aimed to determine differences in top-down and bottom-up control of attention using hyperpolarizing optogenetics in monkeys. Macaques were trained to covertly detect a random dimming of a small target occurring with equal probability at one of four locations in each quadrant while distractors were presented in the other quadrants. Target location was cued either using a change in color of the target (bottom-up) or the fixation point (top-down, symbolic cue for each quadrant), respectively. After the cue, a delay period occurred during which the distractors dimmed, which had to be ignored by the animals. After a random delay period, the monkeys had to indicate the dimming of the target with a manual response in order to obtain a reward. Monkeys were trained to perform this covert spatial attention task while bottom-up and top-down cued trials were randomly presented. Consistent cue-dependent accuracy and reaction time effects were observed in unperturbed animals, as well as relatively small differential fMRI activations between bottom-up versus top-down cued trials in frontal, parietal and visual cortex (N =3, see Balan et al. SfN 2015).
Guided by the fMRI maps, we injected an AAV2/5-CaMKII-Jaws-KGC-eGFP-ER2 viral vector in specific task-driven compartments of both LIP and FEF. After 6 weeks, we aimed to focally reduce neuronal activity in these task-driven foci. Optogenetic inactivation using red light (635nm) was focused on the cue period of randomly selected bottom-up or top-down cued trials, both during fMRI and electrophysiology. For light delivery, we used an optic fiber fixed to a microdrive either with (for electrophysiology and behavior) or without (for fMRI and behavior) electrode.
Preliminary results from the LIP inactivation sessions showed mainly decreased but not completely abolished neural responses and decreased fMRI activity within the targeted section of LIP. During task performance, we also found a mixture of optogenetic-induced decreased and increased fMRI activity throughout nodes of the attention network. Furthermore, we observed decreased behavioral performance during short inactivation of the cue-period, which either affected the bottom-up or the top-down cued trials but rarely both together when the same location was inactivated. These results show that ultra-short reversible inactivation of LIP only

during the cue period can affect top-down and bottom-up driven covert spatial attention behavior, as well as local activity and network dynamics.

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Poster

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Title: The anatomy of the white matter pathways connecting primate dorsal and ventral attention control areas

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Abstract: The cerebral cortex comprises many areas involved in visual attention processing. Classical studies identify the parietal and frontal cortices as two major sources of attentional signals. Recent results highlighted that an area in the temporal cortex also plays a crucial role in visual spatial attention both in humans and monkeys (Stemmann et al., *in preparation*). The discovery of this ventral attentional-control area posits new important questions about the network organization of the primate attention. We're currently asking how dorsal and ventral attention control areas communicate in the two species by combining functional MRI localization with diffusion MRI. In the monkey (*Macaca mulatta*), we acquired high-resolution diffusion MRI (250 x 250 x 250 μm). We used probabilistic tractography to estimate the trajectories of different temporo-parietal pathways. We analyzed the data using constrained spherical deconvolution and an ensemble of tractography methods (Takemura et al., 2016). We optimized the tractography results and tested the statistical evidence for white matter connections

between the ventral and dorsal attention regions by using Linear Fascicle Evaluation methods (Pestilli et al., 2014). We successfully identified white matter pathways connecting the established dorsal attentional maps and the newly reported ventral map. We speculate that this fascicle might be crucial for transmitting attentional signals between ventral regions that encode object properties, including form and identity, and dorsal regions that map spatial information.

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Poster

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Title: A cluster of conspicuity representations for eye fixation selection

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Abstract: A computational explanation of how visual attention, interpretation of visual stimuli, and eye movements combine to produce visual behavior seems elusive. Here, we focus on one component: how selection is accomplished for the next fixation. The popularity of saliency map models drives the inference that this is solved, but we argue otherwise. We advocate for a cluster of complementary, conspicuity representations to drive fixation selection, modulated by task goals and fixation history. This design is constrained by the architectural characteristics of the visual processing pathways, specifically, the photoreceptor distribution in the retina, the pyramidal architecture of the visual pathways, and the poor representation of the visual periphery in the late stages of the visual pathways. Added to these are constraints due to the inappropriateness of an early attentional selection strategy for complex stimuli (eg., non-target displays, figure-ground not easily separated, etc.). Together, these factors led us to a hybrid method that combines early and late selection, i.e., feature and object-based attentional selection. We incorporate attentional microsaccades, saccades and pursuit eye movements into a unified

scheme where true covert fixations (zero eye movement) might only be appropriate if the target of a new attentional fixation is represented in the retina at a resolution sufficient for the task. Finally, elements of a visual working memory structure are included that link fixations across space and provide a means for extracting details of an attentional fixation and communicating them to the rest of the system. These elements combine into a new strategy for computing fixation targets.
Using this new fixation controller, we show results that not only out-perform saliency models with respect to human fixation patterns, but also match very well with human saccade amplitude distribution patterns. Perhaps most importantly, it provides a substrate for a richer exploration of how attention and eye movements are related than possible with other models because it is explicitly designed to be an integrative framework. It is a fully computational framework that can be tested with real images (and image sequences) with no hidden representations or statistically inscrutable elements, generating fixations that can be fully analyzed and providing the full sequence of representations than can also be inspected, analyzed and used to drive experimental testing. It is certainly the case that the specific representations presented will require many refinements; however, the framework offers the ability to understand why those refinements will be needed.

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Poster

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Title: Development of oculo-feedback system: Manipulation of microsaccade generation

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Abstract: We invented an oculo-feedback system that may modify mental states associated with specific eye movements by manipulating their oculomotor properties. Among many kinds of eye movements, we focused on microsaccades (MSCs) that are involuntarily evoked, and known to relate to spatial attention in human, envisioning that we can modify attentional states by

manipulating MSC rate via oculo-feedback. In the proposed oculo-feedback system, MSCs are automatically detected in real-time from eye positions measured binocularly with a video oculography (EyeSeeCam) running on a MacBook, by using a deep convolutional network (DCN). The DCN was configured and pre-trained to dissociate MSCs and non-MSC eye movements from eye position traces (MSC detection rate > 94 %). When a MSC is generated in a specified time window, sensory feedback is given to the subject to reinforce the involuntary behavior. To test the proposed system in manipulating MSC rate and attentional states, we conducted the following experiment. A subject wearing EyeSeeCam sat in a dark room at 45 cm from a 29-inch PC monitor with his/her head fixed. The subject was instructed to look at the first visual cue (a cross with 0.73 deg of visual angle) when it appeared at the center of the monitor. After a random delay between 1.5 and 2.5 sec it was replaced by the 2nd visual cue (left or right arrow head) that told with 80 % accuracy about the direction of a visual target appearing after a random interval between 2.0 and 2.5 sec. The subject was instructed to make a saccade to (pro-saccade condition), or away from (anti-saccade condition) the visual target as soon as it was displayed at either side of the screen simultaneously with disappearance of the 2nd visual cue. After making a pro- or anti-saccade, visual and audio feedback was given by playing an animation in which a circle was enlarged gradually with moderate beep sound, if a MSC had been generated in a 0.2 sec window set at 0.7 sec after the onset of the 2nd visual cue. In one session, this procedure was repeated until the circle reached to the largest size, usually taking about 8 min. Three sessions were conducted a day with a few min of rest, and the subjects underwent 4 days of experiment. Two subjects who participated in the experiment under pro-saccade condition knew their MSCs were evaluated, but did not know about the time window. Results from both subjects showed the numbers of MSC generation in and after the time window progressively increased as they experienced more oculo-feedback sessions, demonstrating that MSC rate is modifiable by the oculo-feedback training presently proposed. Results from the anti-saccade condition, and reaction time to saccade initiations will be presented.

Disclosures: **J. Emoto:** None. **Y. Hirata:** None.

Poster

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Title: Automatic spread of the attentional field from a spatial cue to an underlying object in primary visual cortex

Authors: *M. EKMAN[1], P. R. ROELFSEMA[2], F. P. DE LANGE[1];
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Abstract: Introduction: Spatial attention is commonly understood as a mechanism to increase the neuronal gain in specific locations in the visual field that are associated with a relevant object. However, studies of single-unit recordings have demonstrated that the effect of spatial attention is not limited to the relevant location but also increases the gain in parts of the object that are outside of the spatial priority map [1]. Here, we sought to use a novel decoding model to reconstruct participants' attentional field from activation patterns in early visual cortex (V1) and thus test, to what extent the attention field spreads from a spatial cue to an underlying object.
Methods: Seventeen healthy individuals underwent functional magnetic resonance imaging (3T fMRI) and population-based receptive field mapping [2]. Subjects received a visual cue to attend to two partially overlapping horseshoe-like objects in the left or right visual field. Two colored dots appeared on the attended objects and also on the unattended objects. During the 'object-condition', subjects had to respond whether the two dots were on the same object, irrespective of the dot color. During the 'color-condition', subjects had to indicate whether the dots have the same color, irrespective of whether the dots were on the same object. Notably, one of the dots had a 90% chance of occurring at the intersection of the objects. Therefore, subjects particularly attended this spatial location. Importantly, if attention is object-based, it will spread from the cued location at the intersection either to the lower or to the upper horseshoe-object, depending on which object is positioned on top.
Results and Discussion: Analysis of reaction times showed that participants responded faster when the two dots were on the same object, compared to when they were on different objects ($F(1,16)=22.02$, $P=3.8 \times 10e\text{-}6$), suggesting attentional enhancement of the cued object. This was confirmed by the fMRI-based attentional field reconstruction, which revealed that the BOLD activity spread from the cued location to the underlying object - and represented the object entirely. Importantly, the spreading was also found for the 'color-condition' when the underlying object was not task relevant, suggesting that attentional object selection operates as an automatic process. In sum, our results suggest that the neuronal mechanisms governing spatial attention are closely intertwined with object-based attention.
References: 1.Wannig et al. (2011). *Nat Neurosci*, 2011. 14(10): p. 1243-4. 2.Dumoulin & Wandell (2008) *Neuroimage*. 39(2): p. 647-60.

Disclosures: M. Ekman: None. **P.R. Roelfsema:** None. **F.P. de Lange:** None.

Poster

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Title: Contrasting salience and attention in the early visual pathway

Authors: *S. POLTORATSKI[1], S. LING[3], F. TONG[2];
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Abstract: The visual system employs a sophisticated balance of attentional mechanisms: individuals can willfully guide attention to serve their top-down goals, but can also notice salient information in the environment outside of their current locus of attention. At its simplest, salience can be defined as a measure of local feature contrast across the visual field: if a local region differs from its surrounding context along one or more feature dimensions, it is deemed salient. Here, we used high-resolution fMRI at 7 Tesla to determine the stages at which modulatory effects of bottom-up salience and top-down voluntary attention emerge along the visual hierarchy, and whether these two processes interact to modulate responses in the human visual system. Observers viewed 3 x 4 arrays of gratings, wherein one of the gratings immediately to the left or right of fixation differed from all other items in the array in orientation (or motion direction). To concurrently investigate the effects of top-down attention, observers were cued to attend to the grating to the left or right of fixation, which was either salient or non-salient. Results revealed reliable additive effects of top-down attention and stimulus-driven salience throughout visual areas V1-hV4, with no evidence of an interactive effect. In contrast, the lateral geniculate nucleus (LGN) exhibited significant attentional enhancement but was not reliably modulated by orientation- or motion-defined salience. We conclude that visual salience emerges in feature-selective populations of neurons at early cortical stages of visual processing, and can be distinguished from effects of top-down spatial attention. The finding that simple salience is computed both within and outside the focus of spatial attention informs our understanding of the neural mechanisms of salience and how visual prioritization strikes a balance between the observer's goals and noteworthy stimuli in the environment.

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Poster

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Title: Feature-tuned normalization modulates spatial attention

Authors: *I. M. BLOEM[1,2], S. LING[1,2,3];
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Abstract: Although attention is known to boost the gain of early visuocortical responses, the neural computations and circuitry underlying these gain changes remain unclear. Recent computational models proposed that attentional modulation arises through interactions with divisive normalization, tipping the balance between excitation and inhibition, resulting in a release from gain control. Normalization strength can be assessed utilizing the feature-tuned, weighted nature of normalization, whereby responses to similar features normalize each other more so than responses to dissimilar features. Previous work from our lab has demonstrated that normalization strength and the ability of attention to boost a stimulus-evoked response are related, suggesting that these two measures potentially share a common mechanism (Bloem, & Ling, 2015). In this study, we directly assessed the role that normalization plays in attentional modulation by manipulating spatial attention while simultaneously measuring normalization strength in early visual cortex. We measured fMRI BOLD responses to stimuli that consisted of two orientation bandpass filtered noise patterns, combined in either collinear or orthogonal configurations. Participants were asked to allocate their covert spatial attention to one of two stimuli appearing simultaneously to the left and right of a central fixation point. Observers performed a demanding probe detecting task on the attended side, detecting and discriminating the location of a visual probe appearing within the stimulus. Consistent with our previous work, we found robust attentional and normalization modulation across early visual cortex (V1-V3). Interestingly, the magnitude of attentional modulation depended on the stimulus configuration: we found larger attentional effects when the orientation content of the stimuli was similar (collinear), compared to stimuli with disparate orientation content (orthogonal). Taken together, our results suggest that a neural population's capability for attentional benefits appears contingent upon normalization, whereby a more strongly normalized population results in greater potential for release from suppression driven by attention.

Disclosures: I.M. Bloem: None. **S. Ling:** None.

Poster

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Support: SFB779-TPA1 DFG

Title: The modulatory impact of global feature-based attention on feedfoward and feedback processing in human visual cortex

Authors: *H. G. GARCIA-LAZARO[1], M. BARTSCH[3], H. STRUMPF[2], M. A. SCHOENFELD[1,3], J.-M. HOPF[2,3];
[1]Dept. of Neurol., [2]Otto-von-Guericke Univ., Magdeburg, Germany; [3]Leibniz Inst. for Neurobio., Magdeburg, Germany

Abstract: Global feature-based attention (GFBA) refers to a selection bias of attended feature values outside of the spatial focus of attention. Studies analyzing GFBA with spatially unattended feature probes have shown that the bias arises from gain modulations in extrastriate visual cortex starting around 200 ms after probe onset. Importantly, these modulations were found to propagate in reverse hierarchical direction, suggesting that GFBA reflects a bias of feedback processing in visual cortex. Other studies have documented that GFBA modulations can appear as early as 100 ms after the feature probe, leading to the conclusion that GFBA reflects a bias of the initial feedfoward sweep of processing. Here we investigate an important, yet untested, explanation of the discrepancy: studies reporting modulations during feedfoward processing used experimental designs with a continuous presentation of the target feature, permitting feature-selective units to remain continuously biased across experimental trials, hence the modulation of the initial feedfoward stage of processing. In contrast, studies reporting modulations during feedback processing used target-onset stimulation requiring to bias feature-selective units anew on each trial. To address the issue we recorded the neuromagnetic brain response in human observers performing two versions of an irrelevant feature-probe experiment. An attended color-target shown in one visual field (VF) was combined with the presentation of an irrelevant color probe in the unattended VF. On the "continuous" version, the target was a continuously presented patch of color smoothly moving through color space in a random way, whereas on the "onset" version the target color was presented in a trial-by-trial manner. In both versions subjects had to count the number of occurrences of the target color. The GFBA effect was assessed by analyzing the brain response to the color probes. We observed that during the continuous version of the experiment, attended-color probes elicited a gain modulation as early as 80-90ms in early visual cortex areas. During the onset version, in contrast, target-color probes elicited extrastriate modulations around 180 ms, but no early effect. These observations together

suggest that for feature attention to modulate the feedforward sweep of processing, a continuously driving input to feature-selective units is required. Without such input, the attended feature bias can only be established with the feedback sweep of processing in extrastriate visual cortex.

Disclosures: **H.G. Garcia-Lazaro:** None. **M. Bartsch:** None. **H. Strumpf:** None. **M.A. Schoenfeld:** None. **J. Hopf:** None.

Poster

531. Spatial and Feature-Based Attention

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 531.23/YY13

Topic: D.06. Vision

Title: Feature-specific attentional selection in human prefrontal cortex

Authors: ***N. P. MLYNARYK**, X. ZHANG, S. JAPEE, L. G. UNGERLEIDER;
NIH, Bethesda, MD

Abstract: When features such as motion or color serve as the targets of visual attention, cortical responses to all stimuli with that feature are enhanced. How feature-based attention relays this effect from attended to unattended locations in the visual field remains unclear. Here we used functional magnetic resonance imaging (fMRI) to investigate the mechanism of this global modulation as human subjects performed motion- (Experiment 1) and color- (Experiment 2) based attention tasks. In Experiment 1, subjects were asked to attend one direction of motion within a display of overlapping upward and downward moving dots (the attended stimulus) on one side of a central fixation point, and ignore a single field of dots moving either up or down (the ignored stimulus) on the other side. In Experiment 2, the attended stimulus was comprised of overlapping fields of stationary red and green dots on one side of a central fixation point, and the ignored stimulus was a single field of either red or green dots on the other side. Results indicated that, in Experiments 1 and 2, early visual areas, parietal areas, prefrontal areas, and subcortical areas, as well as the respective sensory areas (MT+ for motion and V4 for color), all showed the classical feature-based attentional effect, with responses to the ignored stimulus significantly elevated when it shared the same feature as the attended stimulus. Further, effective connectivity analysis showed that feature-similar gain modulation outside the attended location was closely associated with feedback from prefrontal areas rather than parietal or subcortical areas. Together, these findings indicate a crucial involvement of the prefrontal cortex in the global effects of feature-based attention.

Disclosures: **N.P. Mlynaryk:** None. **X. Zhang:** None. **S. Japee:** None. **L.G. Ungerleider:** None.

Poster

531. Spatial and Feature-Based Attention

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 531.24/YY14

Topic: D.06. Vision

Title: The color of distraction: the influence of distractors on global color-based attention

Authors: ***M. V. BARTSCH**[1], S. E. DONOHUE[2], Z. PASSAND[1], M. A. SCHOENFELD[1,2], J.-M. HOPF[1,2];
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Abstract: Attention to color is not confined to the spatially attended location, but can operate throughout the whole visual field, with this spatially-independent selection often referred to as global color-based attention (GCBA). Most studies have documented GCBA by comparing the neural response to task-relevant colors to that of irrelevant distractor colors. Key to this comparison is the nature of the distractor (i.e., if it were previously a target color, never a target color (i.e., neutral), and where in the visual field it is presented). As no previous study has taken all of these factors into consideration, including what may be an appropriate baseline for comparison, it is unclear if the previously-observed modulations underlying GCBA reflect the attenuation of distracting colors or, alternatively, the relative enhancement of attended colors in the focus of attention (FOA). Here, we address the issue by analyzing the effect of different types of distractor colors, serving as baseline conditions to evaluate the relative direction of modulations. The general experimental setup follows (Bartsch et al. 2015). Subjects performed a simple color/orientation discrimination task while effects of GCBA were assessed by analyzing the electromagnetic (EEG/MEG) brain response to color probes presented outside the FOA, as a function of whether they matched the currently-attended color inside the FOA. We extended the paradigm by presenting the target together with a distractor color that was neutral and never served as a target color (neutral distractor, ND) or a distractor color that was irrelevant on a given trial block but served as a target on other blocks (relevant distractor, RD). Effects of those distractor colors were assessed with color probes outside the FOA. We observed that the type of distractor (RD or ND) presented with the target color did, indeed, influence effects of global color-based selection. Specifically, while the GCBA response to the attended color was enhanced when compared to the both types of distractor colors, this effect was bigger when compared

against relevant distractors. Additionally, the comparison of relevant vs. neutral distractors revealed global color-based modulations in early visual cortex. This suggests the importance of both implicit and explicit task-relevance in determining what is selected by GCBA, and underscores the importance of having a neutral condition with which to compare such modulations. Together, these observations show that the nature of distractor color determines the operation of GCBA in a qualitative way.

Disclosures: **M.V. Bartsch:** None. **S.E. Donohue:** None. **Z. Passand:** None. **M.A. Schoenfeld:** None. **J. Hopf:** None.

Poster

531. Spatial and Feature-Based Attention

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Program#/Poster#: 531.25/ZZ1

Topic: D.06. Vision

Support: NIH Grant 5T32 EY007031

NIH Grant F32 EY025121

Title: Effects of attention on contrast response functions in foveal visual cortex

Authors: ***R. MILLIN**, M.-P. SCHALLMO, A. V. FLEVARIS, G. M. BOYNTON, S. O. MURRAY;
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Abstract: Foveal vision is used for most tasks that involve analysis of visual form because of its high acuity. Consequently, attention is usually directed to the center of gaze. Despite the fovea's importance to everyday vision, basic visual processes such as neural contrast responses and attention have not been explored for the cortical representation of the fovea because of methodological challenges. Thus, it is unknown to what extent results obtained in the visual periphery will generalize to the fovea. We used fMRI to measure the effect of attention on the fMRI response to image contrast in foveal and peripheral vision. For the first experiment, BOLD measurements were made while subjects viewed oriented gratings and indicated the direction of an orientation change (clockwise or counterclockwise) of either a foveal (width 1 deg) or peripheral (width 3.5 deg, centered at 6 deg) grating. Foveal and peripheral stimuli were presented simultaneously in a blocked design at 5%, 25%, 50%, and 98% contrast. We then performed a second experiment using an event-related design with the same stimuli and tasks as Exp. 1. Cued and un-cued trials with 0% contrast were also included to assess baseline shifts.

The effects of attention on the BOLD response to foveal stimuli were qualitatively different than to peripheral stimuli. Attention to peripheral stimuli produced the commonly observed baseline shift across all contrasts (Buracas and Boynton 2007, Murray, 2008). Attention to the foveal stimulus, however, shifted the contrast-response function from distinctly monotonic to nearly flat. These results from foveal stimuli call into question the generality of conclusions made about the mechanisms of attention based on studies with peripheral stimuli.

Disclosures: **R. Millin:** None. **M. Schallmo:** None. **A.V. Flevaris:** None. **G.M. Boynton:** None. **S.O. Murray:** None.

Poster

531. Spatial and Feature-Based Attention

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 531.26/ZZ2

Topic: D.06. Vision

Support: Leverhulme Trust

Title: Parietal alpha oscillations mediate the attentional shift between different targets in uni- and multimodal paradigms

Authors: ***R. SOKOLIUK**[1], K. J. MULLINGER[4], S. D. MAYHEW[2], K. M. AQUINO[4], R. SANCHEZ PANCHUELO[4], S. T. FRANCIS[4], S. O. HANSLMAYR[3];
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Abstract: A continuous stream of sensory information enters the human brain during daily life. Directing our attention facilitates selecting specific information and inhibiting processing of unimportant information. Recent work has focused on the role of alpha (8-13Hz) oscillations in attention, whereby an increase in alpha power leads to an inhibition of brain areas responsible for processing “to-be-ignored” information. While most previous studies focused on the influence of alpha oscillations within one modality, we were interested in elucidating a more global picture of alpha modulation in attention. Do uni- and multimodal attention paradigms share the same neural source of inhibitory alpha power? How does the neural pattern of alpha modulation change with increasing and decreasing attention? Two different paradigms were used: either attention was divided between the right and left visual hemifields to different degrees (40/60%, 20/80%, 0/100%), or between the visual and somatosensory modalities (40/60%, 0/100%). Each trial

started with a visual cue showing different percentages, to indicate the likelihood with which a target would appear on each side or sensory modality. In the visual paradigm, participants had to identify the orientation of Gabor patterns. In the multimodal visuo-somatosensory paradigm, participants had to discriminate between low and high frequency visual and somatosensory stimuli. The same 16 participants took part in both paradigms whilst high density EEG (128 electrodes) data were recorded. EEG data analyses were performed on the pre-stimulus interval, when subjects were directing their attention towards a specific side or modality. Beamforming analyses were performed on these data to localize the attention modulation effects. Both paradigms, showed a decrease of the mean alpha power during the pre-stimulus period over sensory specific brain regions that process the attended stimuli and an increase over regions processing "to-be-ignored" stimuli. The visual attention task further showed increasing lateralisation of alpha power between the hemispheres with increasing degree of attention, suggesting an effect of cue percentage. In both paradigms, the global source of alpha oscillations was identified in the parietal cortex, suggesting that the observed active inhibition by alpha oscillations is mediated by the same mechanism whether attention is divided spatially in one modality or between two distinct modalities.

Disclosures: **R. Sokoliuk:** None. **K.J. Mullinger:** None. **S.D. Mayhew:** None. **K.M. Aquino:** None. **R. Sanchez Panchuelo:** None. **S.T. Francis:** None. **S.O. Hanslmayr:** None.

Poster

531. Spatial and Feature-Based Attention

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Program#/Poster#: 531.27/ZZ3

Topic: D.06. Vision

Title: Mechanisms of spatial versus non-spatial, modality-based attention

Authors: *D. BALDAUF[1], R. DESIMONE[2];
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Abstract: FMRI and magnetoencephalography (MEG) were used in the same subjects to compare top-down attentional networks for spatial and non-spatial attention. Attention to spatial locations and sensory modality (auditory versus visual) caused enhanced evoked responses and BOLD signals in early sensory areas. Multiple voxel pattern analysis (MVPA) of FMRI data revealed spatial and non-spatial (modality-based) control networks in superior-frontal and inferior-frontal cortex, respectively. FMRI-guided MEG recordings showed similar coherent oscillations between these two frontal control networks and sensory sites according to the

attentional cue, which were enhanced by attention. Our results suggest that different prefrontal structures are sources of top-down biasing signals for spatial and non-spatial attention, and these structures interact in a similar fashion with posterior sensory areas during sustained attention.

Disclosures: D. Baldauf: None. **R. Desimone:** None.

Poster

531. Spatial and Feature-Based Attention

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Topic: D.06. Vision

Support: NIH Grant MH094258

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Caltech Conte Center

Title: Modulation of human medial frontal and amygdala neurons by task demands and attention.

Authors: *J. MINXHA[1], R. ADOLPHS[1], A. MAMALAK[2], U. RUTISHAUSER[2]; [1]Caltech, Pasadena, CA; [2]Neurosurg., Cedars Sinai Med. Hosp., Los Angeles, CA

Abstract: Visually tuned neurons are prominent in areas of the anterior temporal lobe, including the amygdala and the hippocampus, but not in the medial frontal lobe. Medial frontal lobe neurons instead encode task-specific parameters. We investigated the effects of task demands and attention on the visual tuning of neurons in the human amygdala and anterior cingulate cortex (ACC). We recorded from 98 neurons in the amygdala and 112 neurons in the anterior cingulate cortex of five neurosurgical patients. In parallel, we recorded eye-movements as the subjects performed three tasks. First, subjects freely scanned an array of eight images each belonging to one of six categories: fractals, flowers, human faces, monkey faces, cars, and fruits. Second, subjects were asked to fixate and identify if images presented in the periphery (6° by 6°, 18° from center of screen) belonged to a particular category (i.e. is it a car, yes or no). The image location was randomly selected from eight possible locations. Halfway through the session, the target category ("task demands") changed to one of the other image categories (i.e. is it a face, yes or no). Third, we presented two images on opposing sides of the screen and provided a cue to instruct subjects which of the two images was relevant to answer the question. In both the second and third task, we asked subjects to maintain fixation at the center of the screen. Performance was high (91± 3 % accuracy). Trials where subjects broke fixation were excluded from analysis.

Here we show three key results. First, in the free-viewing task, amygdala neurons were visually tuned (n = 30/98, 1x4 ANOVA, aligned 220ms post-fixation, $p<0.05$). In contrast, this was not the case in the ACC (n=6/112). Secondly, in tasks 2 and 3, we also found strong visual-category tuning in the amygdala (n=18/89 in task 2, n=15/35 in task 3, measured with 1x4 ANOVA, 0-500ms post stimulus onset). Thus, neurons were sensitive to where attention was deployed rather than to where the eyes fixated. Thirdly, we found that task demands were strongly encoded only in ACC neurons: both target category (n=35/78, 1x2 ANOVA, 0-500ms post stimulus onset, $p<0.05$) as well as the cued location (n= 8/15, 1x2 ANOVA, 0-500ms post cue onset, $p<0.05$) was encoded. Together, these results show that (i) visually tuned amygdala neurons are sensitive to both covert and overt attention, and that (ii) there is a functional separation between medial-frontal lobe and anterior temporal lobe in representing visual-stimuli and task demands. This suggests that studying the functional interactions between the amygdala and the anterior cingulate cortex is a fruitful approach to determine the dynamic implementation of task demands.

Disclosures: **J. Minxha:** None. **R. Adolphs:** None. **A. Mamalak:** None. **U. Rutishauser:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

Location: Halls B-H

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Topic: D.09. Multisensory Integration

Support: Sloan Foundation

Title: Multi-finger cue combination depends on hand proprioception

Authors: *M. RAHMAN, J. M. YAU;
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Abstract: Our hands are remarkably dexterous organs that we use to sense and manipulate objects. Hand dexterity depends on the fact that the somatosensory system uniquely contains a deformable sensory sheet - The relative positions of the cutaneous receptors in 3D space depend on the conformation of the hand. The haptic perception of 3D objects clearly requires combining cutaneous signals about the local events on multiple fingers with proprioceptive signals about hand postures. Critically, how proprioception and cutaneous sensing interact is poorly understood. In a series of behavioral experiments we investigated how hand posture influences the perception of vibrations over multiple fingers, i.e., multi-finger touch. For a measure of multi-finger touch, we first established how vibration information delivered simultaneously to

two fingers is combined. Preliminary results indicate that ignored vibrations delivered to one finger systematically influence the perception of attended vibrations delivered to a different finger. These multi-finger interactions depend on relative finger position: fingers spread further apart interact less than fingers held closely together. Lastly, we found that multi-finger interactions occur similarly within and across hands. To better understand these perceptual effects, we developed a probabilistic model capturing interactions between proprioception and multi-finger touch. We modeled cue interactions over multiple fingers as a linear combination of the probabilistic sensory representations of the attended and ignored cues. In our model, proprioception determines cue weighting - increasing the separation between fingers reduces the weighting of the ignored cue and increases the weighting of the target cue. These early results imply that the nervous system accounts for the relative positions of multiple fingers when combining sensory cues over them. Conceivably, the interactions between proprioception and multi-finger touch reflect the statistical regularities in how we sense and manipulate objects using our hands.

Disclosures: M. Rahman: None. **J.M. Yau:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

Location: Halls B-H

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Topic: D.09. Multisensory Integration

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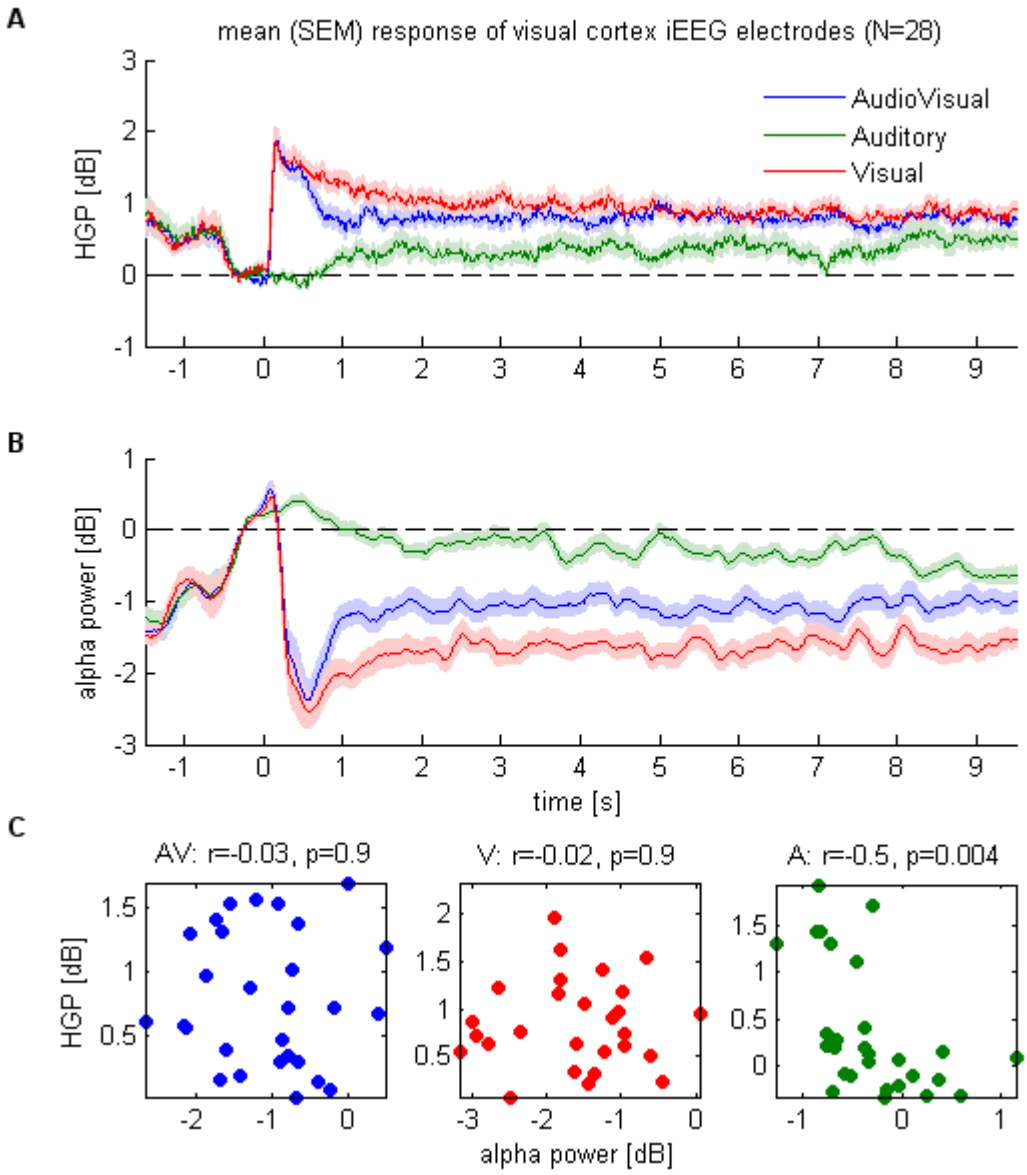
Title: Human visual cortex responses to naturalistic auditory and visual speech

Authors: *P. MEGEVAND[1], M. R. MERCIER[2], D. M. GROPPE[3], E. ZION GOLUMBIC[4], N. MESGARANI[5], C. E. SCHROEDER[6,7], A. D. MEHTA[3];
[1]Neurol. Dept., Geneva Univ. Hosp., Geneva, Switzerland; [2]Ctr. de Recherche Cerveau et Cognition, Univ. Paul Sabatier, Toulouse, France; [3]Neurosurg., Hofstra Northwell Sch. of Med. and Feinstein Inst. for Med. Res., Manhasset, NY; [4]Gonda Brain Res. Ctr., Bar Ilan Univ., Ramat Gan, Israel; [5]Electrical Engin., [6]Neurosurg., Columbia Univ., New York, NY; [7]Cognitive Neurosci. Lab., Nathan S. Kline Inst., Orangeburg, NY

Abstract: Speech is multisensory by nature: we must move to speak, and these movements, which are visible to our interlocutors, complement the auditory information transmitted by the voice. Cerebral cortex is also essentially multisensory: even primary sensory cortices are sensitive to stimuli from modalities other than their own. Here, we explored how human visual cortex processes naturalistic audiovisual speech using intracranial EEG (iEEG) in patients with drug-resistant epilepsy. We recorded cortical responses to 10-s long stories consisting of naturalistic speech (modalities: AV, audiovisual; V, visual only; A, auditory only). iEEG electrodes were localized by co-registering pre- and post-implantation MRI and CT scans. We quantified cortical responses to speech stimuli by computing power in the alpha (8-13 Hz) and high-gamma frequency ranges (HGP, an index of local neuronal firing; 70-170 Hz). We defined visual cortex as those electrodes that lay in the occipital lobe and showed increased HGP to V speech stimuli, compared to the pre-stimulus baseline. Visual cortex responded to V and AV speech with a robust and sustained increase in neuronal activity, which dipped lower for AV than for V speech between 600 and 1200 ms into the stimulus before reaching a stable plateau (Figure, A). In contrast, HGP increased in delayed and crescendo fashion to A speech, rising above baseline about 1 second into the stimulus. Alpha power dropped in marked and sustained fashion in response to V and AV speech (more so for V than AV speech), whereas it slowly decreased in response to A speech (Figure, B). There was a significant negative correlation between HGP and alpha power in response to A speech, but not to V and AV speech (Figure, C). Our findings extend those of Schepers and colleagues (Cereb Cortex 2015), who recorded iEEG responses to single-word stimuli. The delayed HGP increase in visual cortex in response to purely auditory speech, and its negative correlation with the reduction in alpha power, suggest that crossmodal sensory inputs modulate neuronal firing in visual cortex through oscillatory mechanisms.



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Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

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Topic: D.09. Multisensory Integration

Support: CIHR Grant 9335

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Title: Haptic object representation in lateral occipito-temporal cortex depends on hand location and gaze direction

Authors: *L. STROTHER[1], Z. ZHOU[1], T. VILIS[2], J. C. SNOW[1];
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Abstract: Human lateral occipito-temporal cortex (LOTC) plays a central role in visual object recognition, and there is growing evidence that LOTC may also play an important role in haptic object recognition. During visual object recognition, LOTC shows gaze-centered (retinotopic) responses in fMRI experiments. The relevant reference frames for LOTC during haptic object recognition are not fully understood. For instance, reference frames for haptic object recognition processes in LOTC may be hand-based, body-based, gazed-based or all three in combination. We used fMRI to test whether or not the neural representation of familiar objects in LOTC depends on the location of the palpating hand with respect to the body. Right-handed participants palpated objects using the right hand, either on the right side of the body or on the left (by crossing the right hand over midline). Objects were always hidden from view, but a mirror enabled subjects to gaze in the direction of the object, or away from it. Our main prediction was that left LOTC would show greater fMRI responses when objects were palpated on the right side of the body as compared to the left, and the opposite for right LOTC. If so, this would parallel the known contralateral bias in LOTC (in both left and right hemispheres) for visually presented objects with respect to the visual field. Our secondary prediction was that fixating in the direction of the palpated object would result in greater fMRI responses in LOTC than looking away, in accordance with prior findings related to visually guided action. In contrast to our main prediction, the magnitude of fMRI responses in left LOTC showed no dependence on the location of the palpated object with respect to the body. Surprisingly, however, fMRI responses in right LOTC were greater for objects palpated on the right (ipsilateral) side of the body as compared to the left. This intriguing result was opposite to our main prediction, which was that LOTC would show greater fMRI responses to objects palpated on the contralateral body side. Related to our second prediction, fMRI responses in right LOTC were greatest when participants palpated objects on the right side of the body and directed gaze toward the object.

Disclosures: L. Strother: None. **Z. Zhou:** None. **T. Vilis:** None. **J.C. Snow:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

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Facility for Education and Research in Neuroscience training grant

Title: Neural basis of the crossmodal correspondence between auditory pseudowords and visual shapes.

Authors: *K. MCCORMICK[1], S. LACEY[1], R. STILLA[1], L. C. NYGAARD[1], K. SATHIAN[1,2]; [1]Emory Univ., Atlanta, GA; [2]RR&D Ctr. for Visual and Neurocognitive Rehabilitation, Atlanta VAMC, Atlanta, GA

Abstract: Behavioral research has demonstrated systematic crossmodal correspondences for many perceptual domains, e.g., between pseudowords and visual shapes bearing protuberances varying from rounded to pointed: the pseudoword “kike” (pronounced kee-kay) is typically associated with pointedness and the pseudoword “lomo”, with roundedness. Such correspondences, termed “sound symbolic”, have been theorized to underlie the origins of human language. The neural mechanisms mediating these correspondences are unknown. Here we used functional magnetic resonance imaging (fMRI) to examine the neural basis of the sound symbolic crossmodal correspondence between auditory pseudowords (“kike”, “lomo”), and pointed/rounded visual shapes. Participants (n=19) were presented with audiovisual stimuli in a block design. Stimuli either congruent or incongruent with respect to the sound symbolic correspondence occurred in separate, interleaved blocks. Participants attended to either auditory or visual stimuli, performing a two-alternative forced-choice on the attended modality. Reaction times (RTs) were recorded (RTc: RT for congruent trials; RTi: RT for incongruent trials). A neural incongruency effect (IE), indexed by greater blood oxygenation level-dependent (BOLD) responses for incongruent trials relative to congruent trials in the attend-auditory condition alone, was observed bilaterally in regions implicated in attention or cognitive control, including the frontal eye fields (FEFs), middle and inferior frontal gyri (MFG, IFG), anterior insula, intraparietal sulcus (IPS), and cingulate cortex. Many of these regions had IEs correlating negatively across individuals with the behavioral IE: (RTi - RTc)/(Rti + RTc); the negative correlation indicates that people with smaller behavioral IEs had stronger BOLD IEs.
Three localizers, run to test proposed explanations for crossmodal correspondences, showed, among voxels with IEs: about a quarter, located in the MFG and IPS bilaterally and the right

anterior insula, were more active when audiovisual stimuli were asynchronous than synchronous; some foci, in the right FEF, IFG and IPS, were active during magnitude estimation; and a few left IFG foci were active during semantic processing. Our findings may reflect multiple neural processes during processing of incongruent sound symbolic stimuli, including deployment of attention and/or cognitive control, multisensory processes, magnitude estimation (e.g. of roundedness or pointedness), and only minimally, semantic mechanisms.

Disclosures: **K. McCormick:** None. **S. Lacey:** None. **R. Stilla:** None. **L.C. Nygaard:** None. **K. Sathian:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

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Program#/Poster#: 532.05/ZZ9

Topic: D.09. Multisensory Integration

Support: FINEP 01.12.0514.00

AASDAP

AACD

Itau Bank

Title: Measuring lower limb peripersonal space in spinal cord injury patients using an audio-tactile stimulation

Authors: **A. ESSIG**[1], S. SHOKUR[2], A. SCHALLER[1], S. GALLO[1], *A. C. DONATI[2,3], G. BAO[2], M. BOURI[1], H. BLEULER[1], M. NICOLELIS[4,5,6];
[1]EPFL, Lausanne, Switzerland; [2]Associacao Alberto Santos Dummont Para Apoio A Pes, AASDAP, Sao Paulo, Brazil; [3]Associação de Assistência à Criança Deficiente, Sao Paulo, Brazil; [4]IIN-ELS - Intl. Inst. For Neuroscienc, Macaiba RN, Brazil; [5]Neurobiology, Biomed. Engineering, Psychology and Neurosci., Duke Univ., Durham, NC; [6]Duke Univ., Duke Ctr. for Neuroengineering, Durham, NC

Abstract: Peripersonal space (PPS) defines the neural representation of the space immediately surrounding our bodies. This representation is essential for us to interact with our surrounding environment. Numerous studies have shown that PPS is plastic and can change, for example, when a subject uses an external tool. Conversely it has been shown that PPS shrinks for amputee patients around their stump. Here, we investigated the PPS for paraplegic patients produced by a

chronic spinal cord injury (SCI). This was done by implementing a new technique based on a combined audio-tactile stimulation to assess PPS boundaries. After repeated measures in seven SCI patients, we identified a smaller PPS around the lower limbs when compared to a group of healthy subjects. The PPS boundary in SCI patients was also less well defined than in healthy subjects. Moreover, we observed that just a few minutes walking using a robotic gait trainer were enough to enhance the PPS of SCI patients.

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Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 532.06/ZZ10

Topic: D.09. Multisensory Integration

Title: Early multisensory comparison and integration in peri-hand space

Authors: ***J. LIMANOWSKI**, F. BLANKENBURG;
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Abstract: To guide the body's actions in the world, the brain needs to integrate information coming from visual and somatosensory systems into a coherent representation. Here we investigated the early phase of such multisensory integration by manipulating the congruence of visual and proprioceptive information about hand position and the congruence of visual and tactile touch locations on the same hand.
We assessed brain activity using functional magnetic resonance imaging (EPI, TR = 2 s, 3x3x3 mm voxel size) while thirteen healthy participants had their right hand placed in a custom rotatable apparatus that was used to change their hand's position passively, and were presented a virtual, photorealistic hand in 3D via stereoscopic goggles in a position corresponding to their real hand. The virtual hand was always in an "anatomically plausible" position, even when incongruent to the real hand's one. We applied simultaneous touches (6 s duration, 14 s rest, jittered onsets) to either the index finger or the little finger on either hand: the virtual hand model's fingers were touched by little rods that moved up and down in an unpredictable rhythm at about 1.5 Hz; the real hand's fingers were simultaneously "touched" by electrotactile impulses. In between stimulations (no virtual hand visible), the position of the participant's real hand was changed passively from palm facing to back facing, or vice versa. Thus each presentation was an unpredictable combination of real and virtual hand position (congruent or incongruent) and touch location (same finger or different finger).

We observed significant ($p < 0.05$, corrected for multiple comparisons) activity increases during visuo-proprioceptive congruence in the bilateral inferior parietal lobe and the left medial superior parietal lobe, and at uncorrected thresholds also in the premotor cortex. The cerebellum and posterior superior temporal sulcus relatively increased their activity during incongruent arm position presentations ($p < 0.001$, uncorrected). We observed significant activity increases during touch applied to the same versus different fingers in the left anterior insula ($p < 0.05$, corrected) and at uncorrected thresholds also in the left ventral premotor and prefrontal cortex, posterior parietal cortex, and lateral occipitotemporal cortex. These activations likely indicate the early phase of multisensory comparison and integration processes that ultimately help construct a coherent body representation. Our results suggest different brain networks involved in processing visuo-proprioceptive position information, and in comparing and remapping visuo-tactile reference frames.

Disclosures: J. Limanowski: None. **F. Blankenburg:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 532.07/ZZ11

Topic: D.09. Multisensory Integration

Support: James S. McDonell Foundation

The Swedish Research Council

Torsten Söderbergs Stifelse

Riksbankens Jubileumsfond

Title: Ownership of a hand increases its visibility in binocular rivalry

Authors: *B. VAN DER HOORT, M. REINGARDT, H. H. EHRSSON;
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Abstract: In binocular rivalry both eyes receive different visual input, which leads to the alternating perception of each eye's input. Binocular rivalry is often used to investigate visual awareness, and the processes that affect it. For example, previous studies have shown that binocular rivalry can be influenced by sensory modalities other than vision, such as tactile, proprioceptive, and vestibular senses. Here, we hypothesized that if one of the competing images is a hand, its visual dominance would be enhanced when participants experience ownership of

that hand. More specifically, the brain favours the visual processing of a hand stimulus if that hand is our own.
To investigate this, we performed two within-subject binocular rivalry experiments in which the image of a hand was presented to one eye and the image of a mask to the other eye. In Experiment 1 (n=30), we manipulated the visual appearance of a touch and the tactile sensation of a touch. The "seen touch" was the presence of a moving ball attached to a stick, presented to both eyes. The "felt touch" was the tactile stimulation of a participant's veridical hand by a similar ball on a rod. In the 'visuotactile condition', the seen touch and the felt touch were synchronous and spatially congruent, which elicits the illusory percept that the hand in view is one's own hand (body ownership). Importantly, this also led to the hand image being more dominant compared to when only the seen touch was present ('visual only condition'), only the felt touch was present ('tactile only condition'), or neither was present ('baseline condition') (vs visual only: $t(29) = 2.54$, $p = 0.017$; vs tactile only: $t(29) = 4.39$, $p < 0.001$; vs baseline: $t(29) = 4.58$, $p < 0.001$). Furthermore, we found that participants that rated stronger feeling of ownership had a larger effect of visuotactile stimulation of the hand (Spearman correlation: $\rho_S = 0.61$, $p < 0.001$). In a second experiment (n=30) we manipulated ownership of the hand directly by rotating the image of the hand. A rotated hand does not allow for the sensation of ownership because it is not spatially congruent with the sensed position of one's veridical hand. Importantly, disrupting ownership of the hand this way, caused the effect of visuotactile stimulation to disappear ($F(1,29) = 6.62$, $p < 0.012$). Summarizing, we found that perceived body ownership can affect visual awareness of the image of a hand in a binocular rivalry paradigm. This finding increases our understanding of the interaction between the representation of our body and the visual system, by showing that our body representation can affect visual processing at the early stages at which binocular rivalry takes place.

Disclosures: **B. Van Der Hoort:** None. **M. Reingardt:** None. **H.H. Ehrsson:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

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Topic: D.09. Multisensory Integration

Support: Grant-in-Aid for JSPS Fellows

Title: Neural substrate of the correspondence between abstract auditory and visual information: an investigation through crossmodal multivariate pattern analysis

Authors: *S. KANAYA[1,2], H. YAMAMOTO[1], H. YAMASHIRO[3], T. MURASE[4], M. UMEDA[4], T. HIGUCHI[4], J. SAIKI[1];
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Abstract: Certain systematic relationships between seemingly unrelated multisensory information are referred to as crossmodal correspondence. For instance, a small figure and a high pitch may be perceived as congruent. In the present study, we performed multivariate pattern analysis (MVPA) on fMRI data to investigate how the correspondence between abstract multisensory information is represented in the brain and its relation to the crossmodal representation of natural objects based on daily experience. Abstract visual and auditory stimuli included four line drawings and four complex sounds, respectively. Visual stimuli included two line drawings consisting of curved lines (curvy figure) and two drawings consisting of straight lines forming an acute angle (sharp figure). Auditory stimuli included two sounds that were judged as congruent with the curvy figures and two sounds judged as congruent with the sharp figures. Natural visual and auditory stimuli included line drawings and the sounds of a fuurin (Japanese wind chime) and a string instrument, each of which had two exemplars. In each trial, one of the visual or auditory stimuli was presented unimodally in a 3T scanner. There were two task conditions. Participants in the no-image group passively observed stimuli and pressed a button when seeing a color change at the fixation point. Participants in the image group were informed of the correspondence between visual and auditory stimuli beforehand, and instructed to image the counterpart when presented with one of the stimuli. In MVPA, we trained a classifier to discriminate 'curvy' vs 'sharp' for abstract stimuli, and 'fuurin' vs 'string instruments' for natural stimuli. The classifier was tested to discriminate unlearned data either intramodally (e.g. training and testing with visual stimuli) or crossmodally (e.g. training with visual stimuli and testing with auditory stimuli). The results of intramodal MVPA revealed that relatively broad regions, including visual and auditory areas, showed reasonably high classification accuracy regardless of the stimulus or group. To the contrary, the results of crossmodal MVPA showed an interaction between stimulus and group. For abstract stimuli, above-chance accuracies were observed in several regions in the parietal and temporal lobes in both groups. For natural stimuli, high accuracies were observed in relatively broad regions in the image group, while almost no areas showed accuracies above chance in the no-image group. These results suggest that the correspondence between visual and auditory abstract information is represented in a different way from the crossmodal representation of natural stimuli in our brain.

Disclosures: **S. Kanaya:** None. **H. Yamamoto:** None. **H. Yamashiro:** None. **T. Murase:** None. **M. Umeda:** None. **T. Higuchi:** None. **J. Saiki:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 532.09/ZZ13

Topic: D.09. Multisensory Integration

Title: Effects of auditory processing on tactile sensory-evoked potentials (SEPs)

Authors: *G. SHEN, P. J. MARSHALL;
Temple Univ., Philadelphia, PA

Abstract: A number of recent behavioral studies examined the interactions between tactile sensation and speech perception. Auditory stimuli have been shown to enhance tactile frequency and intensity perception, and tactile stimulations of articulatory areas can influence phonological perceptual judgment. However, the mechanism underlying those observed interactions is still unclear. Is this cross-modal interaction restricted by temporal distance and semantic congruency, or is it a global type of modulation where one sensory input modulates the overall excitability of sensory regions? The current study examines temporal and congruency effects in somatosensory-auditory interactions using tactile stimulation and meaningful sounds, focusing on the somatosensory evoked potentials (SEPs) and mu rhythm fluctuations elicited to these events. Participants were presented with speech syllables (/pa/, /ka/) and finger snapping sounds followed by tactile stimulation to either their lower lip or their right middle finger in a random order. Intervals between the two sensory stimuli were set to 600ms, 200ms, or 0ms (simultaneous presentation). EEG signals were recorded as participants received auditory and tactile stimuli in pairs, and attended to the tactile sensation by detecting double-pulse tactile stimuli that were randomly mixed with single-pulse stimuli. We observed that early contralateral tactile SEPs in response to lip stimulation were modulated by speech processing only when intervals between the two events were 200ms or shorter. Preliminary results showed that /pa/ sounds significantly increased lip SEP amplitude as early as 50ms post-stimulus compared to /ka/ and finger snap sounds, while no differences were found in finger SEPs after the three different sounds. The results suggest that perception of a bilabial speech sound, /pa/, can enhance sensory excitability of the corresponding articulator. The findings of this novel auditory/lip-tactile paradigm indicate that somatosensory-auditory interaction is dependent on the critical temporal distance and congruency between the phonetic nature of speech sounds and the location of somatosensory stimulation. The findings may also shed light on the ongoing debate on the sensorimotor involvement in speech perception.

Disclosures: G. Shen: None. **P.J. Marshall:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

Location: Halls B-H

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Topic: D.09. Multisensory Integration

Support: U.S. Army Research Laboratory under Cooperative Agreement Number W911NF-10-2-0022

Title: Neural correlates of visual-haptic decision making in a texture discrimination task

Authors: *I. DELIS, P. SAJDA, Q. WANG;
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Abstract: The signals delivered by different sensory modalities provide us with complementary information about the environment, which is then used to make perceptual decisions. Whereas the importance of multisensory integration for perceptual decisions has been appreciated in recent years, its underlying neural mechanisms still remain elusive. Importantly, the processing of multisensory information requires the interaction of multiple brain areas over time. Here we investigated the neural underpinnings of visual-haptic integration during performance of a two-alternative forced choice (2AFC) reaction time (RT) task. We asked human subjects to discriminate the amplitude of two texture stimuli a) using only visual (V) information, b) using only haptic (H) information and c) combining the two sensory cues (VH), while electroencephalograms (EEG) were recorded. Subjects' task performance - reflected in discrimination accuracy and reaction time - improved when both types of sensory signals were available. Modelling of the behavioral performance using a hierarchical drift diffusion model (HDDM) revealed that in the VH condition subjects accumulated evidence near-optimally across modalities. Single-trial analysis of whole-scalp EEG signals identified neural components discriminating the presented stimuli and/or the subjects' choices.

Disclosures: I. Delis: None. **P. Sajda:** None. **Q. Wang:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

Location: Halls B-H

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Topic: D.09. Multisensory Integration

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Title: Prestimulus oscillatory activity can provide predictive value for false perception of somatosensory stimuli

Authors: *K. YOSHINAGA[1,2], M. MATSUHASHI[3,5], T. HANAKAWA[1], T. MIMA[3,6], H. FUKUYAMA[3], R. TAKAHASHI[2], A. IKEDA[4];
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Abstract: [Introduction]In a discrimination task, subjects sometimes report false detection of a stimulus (false alarm) in stimulus-absent trials, but the mechanisms of the false perception remain enigmatic. Recently, pre-stimulus oscillatory activity has been shown to affect subsequent human behaviors such as subjects' ability to detect a near-threshold stimulus in a discrimination task. In the present study, we tested a hypothesis that pre-stimulus oscillatory activity could predict perception of stimuli without physical exposure to stimuli. [Method]Healthy volunteers participated in the study. The participants were asked to detect a somatosensory stimulus (electric stimulation of the right digital nerve at a subject-specific near-threshold intensity level) given simultaneously with visual stimulus (LED stimulus much above the detection threshold). The somatosensory stimulus was delivered in a half of the trials ("S+V" trial) and only a visual stimulus was given in the rest ("V" trial). Participants sometimes reported detection of a somatosensory stimulus in the "V" trials, yielding "false alarm" (FA) and "correct rejection" (CR) trials. Ongoing brain activity was recorded with the 306-channel magnetoencephalography. We calculated pre-stimulus power differences between the FA and CR trials. To investigate phase effects, we evaluated a degree of deviation of phase distribution in each condition using Mardia-Watson-Wheeler test. [Result]In the power analysis, we found

that pre-stimulus power in the left central region was stronger in the CR trials than in the FA trials, suggesting an up-state of somatosensory areas in the FA trials. This power difference was identified in the alpha and/or beta frequency range at an individual level. In contrast, we found stronger pre-stimulus alpha oscillations in the parieto-occipital region in the FA trials than in the CR trials, suggesting a down-state of visual areas in the FA trials. In phase analysis, however, we found no remarkable phase deviation in either condition. [Conclusion]This result suggests that the combination of up-state of the somatosensory system and down-state of the visual system may lead to a generation of false perception in a somatosensory discrimination task.

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Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

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Topic: D.09. Multisensory Integration

Support: EY014645 to Ione Fine

EY023268 to Fang Jiang

Title: Cross-modal motion processing after sight recovery

Authors: *F. JIANG[1], A. N. SCURRY[2], I. FINE[3];
[1]Psychology, Univ. of Nevada, Reno, Reno, NV; [2]Psychology, Univ. of Nevada Reno, Reno, NV; [3]Psychology, Univ. of Washington, Seattle, WA

Abstract: Recently we showed that hMT+ responses to auditory motion are associated with subjects' decisions about auditory motion direction in early blind individuals (Jiang et al., 2014). Here, we examine whether these cross-modal motion responses arise 'de novo' or co-opt residual visual architecture, by comparing visual and auditory responses to motion in a sight recovery subject, MM. MM acquired vision in adulthood after becoming blind at age three. Despite severe losses in acuity, MM has no known deficits in his ability to process visual motion and shows normal hMT+ responses to moving dots. However, as a result of being blind early in life, his hMT+ also shows robust cross-modal responses to auditory motion (Saenz et al. 2008).
We examined joint tuning for motion direction across visual and auditory stimuli in MM using both multivoxel pattern classification and fMRI adaptation. The direction of auditory motion could be successfully classified based on the pattern of BOLD responses within bilateral hMT+

to a visual motion stimulus, and vice versa, indicating congruence in architecture between auditory and visual direction motion tuning within hMT+. Furthermore, significant adaptation effects were found in the right hMT+ when using a visual adaptor - responses to auditory motion were weaker when auditory motion was in the same direction as the visual motion adaptor, suggesting shared neuronal tuning across vision and audition. Our results show that in area hMT+ the directionally tuned auditory motion responses induced by early blindness share common architecture with residual visual motion responses.
References cited:
Jiang, F., Stecker, G.C., Fine, I. (2014). Auditory motion processing after early blindness. Journal of Vision, 14(13):4.
Saenz, M., Lewis, L.B., Huth, A.G., Fine, I., Koch, C. (2008). Visual motion area MT+/V5 responds to auditory motion in human sight-recovery subjects. Journal of Neuroscience, 28 (20), 5141-5148.

Disclosures: F. Jiang: None. **A.N. Scurry:** None. **I. Fine:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

Location: Halls B-H

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Topic: D.09. Multisensory Integration

Title: Periferal cooling of hypothalamic pituitary axis in humans

Authors: *B. BARRERA-MERA;
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Abstract: Selective cooling of the human forebrain have lead to relief severe (Barrera-Mera 2013 Neurosci. Abst.) or slight mental maladies (Barrera -Mera, 2014;2015 Neuroci. Abst.) Such effects of cooling however failed in some kind of patients as those addicted to inhalants because they are hard to convince themselves about the benefits of such innocuous and inexpensive remedy In such a way now we find other manners to improve mental and corporeal human discomfort by using the benefits of cooling selectively the peripheral components neuro-visceral systems of elderly subjects.

Disclosures: B. Barrera-Mera: None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

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Topic: D.09. Multisensory Integration

Support: DFG Grant KE1828/2-1

Title: Oscillatory EEG activity in the ventriloquist illusion

Authors: ***M. KAISER**, D. SENKOWSKI, J. KEIL;
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Abstract: In our environment we are faced with a vast amount of stimuli that we integrate across the different sensory modalities to a unified perception. During this process, stimuli can exert cross-modal influence to modulate perception. One prominent example for this is the ventriloquist illusion, where sound localization is shifted in the direction of a concurrent visual stimulus, exemplifying cross-modal influence from the visual to the auditory modality. Recent ERP and fMRI studies have shown that the ventriloquist illusion is linked with activity asymmetries between left and right auditory cortices. Moreover, studies using other multisensory illusion paradigms have consistently shown pre- and poststimulus modulations in alpha, beta and gamma band oscillations in sensory and traditional multisensory integration areas related to illusory perception. Thus far, it is unknown whether cross-modal influence in the ventriloquist illusion is also reflected in oscillatory activity. Additionally, the role of neural oscillations prior to stimulus onset on the subsequent perception in this illusion is unknown.
In this study, we aimed to assess these questions using high-density EEG recordings from healthy subjects. We compared oscillatory activity between trials in which the illusion occurred and trials with veridical auditory localization. That is, we compared oscillatory activity between trials with invariable physical stimulus properties, but variable perception. We performed time-frequency analysis focusing on effects in the alpha, beta and gamma bands. Dependent-samples t-tests with Monte Carlo randomization and cluster-based correction for multiple comparisons were used for statistical analysis. Source space activity was reconstructed using beamforming techniques.
Our data indicate asymmetrical poststimulus oscillatory activity across both auditory cortices in illusion, but not in no-illusion trials. Furthermore, oscillatory activity in the visual cortex differed between illusion and no-illusion trials. Our findings indicate that the ventriloquist illusion is mediated by early oscillatory activity in sensory areas. These results have implications for the question whether the ventriloquist illusion reflects a perceptual effect rather than a result of later attributing the origin of the sound towards the visual stimulus. Our findings corroborate and

extend previous findings on the cortical mechanisms underlying the ventriloquist illusion and suggest that neural oscillations play an important role in this illusion.

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Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

Location: Halls B-H

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Topic: D.09. Multisensory Integration

Support: U.S. Army Research Laboratory Cooperative Agreement Number W911NF-12-2-0019

U.S. Army Research Laboratory Cooperative Agreement Number W911NF-10-2-0022

Title: Network flexibility during multisensory integration of real-world events.

Authors: *G. LIEBERMAN[1,2], J. O. GARCIA[1], D. S. BASSETT[2], M. J. TARR[3], J. M. VETTEL[1,4,2];
[1]U.S. Army Res. Lab., Aberdeen Proving Ground, MD; [2]Bioengineering, Univ. of Pennsylvania, Philadelphia, PA; [3]Psychology, Carnegie Mellon Univ., Pittsburgh, PA; [4]Psychology, Univ. of California, Santa Barbara, CA

Abstract: Real-world events produce both auditory and visual signals that combine to form integrated perceptual experiences. Research suggests that binding perceptual signals from different senses depends on low-level cues such as temporal/spatial structure and high-level cues such as semantic labeling (Doehrmann & Naumer, 2008; Keetels & Vroomen, 2012). Importantly, these cues may rely on separable but interconnected networks (Tsilionis & Vatakis, 2016). Here, we use community detection to investigate how making explicit judgements about temporal synchrony and semantic congruency modulates brain activity in a distributed multisensory network. Fourteen volunteers participated in an fMRI study and viewed videos of real-world events that included multiple, discrete impacts (e.g., a pencil tapping, a maraca shaking). Videos were manipulated such that the timing and/or the semantics of the impacts could be congruent or incongruent in a 2x2 factorial design. For semantically incongruent conditions, the audiovisual movies contained the sound track from one event (e.g., water splashing) and the visual track from another event (e.g., clapping). For temporally asynchronous conditions, the visual and auditory impacts were offset by 500 milliseconds. Participants completed three tasks: passively viewing the stimuli, judging temporal synchrony, and judging semantic congruency.

Using the passive viewing task, we identified four network communities subserving audiovisual integration, and we compared how network flexibility varied within these communities when participants made explicit congruency judgments. During semantic judgments, a significant decrease in flexibility was detected in the left superior parietal lobe (FDR $q < 0.05$), driven by enhanced network allegiance between this region and nodes located bilaterally within the frontal cortices. Additionally, this difference in flexibility was pervasive across the brain: more communities were identified for judgments of temporal synchrony than for judgments of semantic congruence ($p < 0.0001$). These results provide some of the first evidence that semantic and temporal judgements differentially recruit dynamic brain networks when audio and visual components of real-world events are integrated within the brain.

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Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

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Topic: D.09. Multisensory Integration

Support: UWE QR grant

Pain Relief Foundation

CIHR Fellowship Award

Title: Neural correlates of a visuo-haptic illusion

Authors: *M. MOAYEDI[1,2], K. THEMELIS[3], T. V. SALOMONS[4], R. NEWPORT[3], J. LEWIS[5,2];
[1]Univ. of Toronto, Toronto, ON, Canada; [2]Univ. of the West of England, Bristol, United Kingdom; [3]Sch. of Psychology, Univ. of Nottingham, Nottingham, United Kingdom; [4]Univ. of Reading, Reading, United Kingdom; [5]The Royal Natl. Hosp. for Rheumatic Dis., Bath, United Kingdom

Abstract: The perception of our body can be easily manipulated, as evidenced by a variety of illusions that change the shape of the body, demonstrating that the manner in which the body is perceived is highly adaptive. These illusions disrupt the body schema – a virtual, dynamic multisensory map of the body within the sensory environment. The illusory disruption of the body schema can be reinforced by simultaneously manipulating two or more sensory channels.

For example, reinforcing a visual illusion with haptic stimulation substantially enhances the robustness of the illusion, and the rates of susceptibility. The combination of visual and tactile information requires multisensory processing and binding in higher order cortical regions, such as the extrastriate body area on the temporo-occipital junction and the posterior parietal cortex. We aimed to identify the neural underpinning of a robust visuo-haptic illusion where the finger is perceived as being stretched in response to being pulled, congruent with visual elongation.
18 healthy (10f, 8m) subjects consented to procedures approved by local ethics committees, and were were naïve to the finger stretch illusion. Subjects underwent fMRI scanning while viewing a digital image of their finger either undergoing a stretch or a sham illusion, where there was no digital alteration of the finger.
Trial-by-trial subjective ratings showed that subjects were more susceptible to the the finger stretch illusion than the sham illusion ($p<0.001$). Functional brain images showed greater brain activity in the mediodorsal thalamus, the mid-cingulate cortex, the extrastriate body area, the ventrolateral prefrontal cortex, the cerebellum and the posterior parietal cortex ($p<0.05$, corrected). These regions are implicated in salience detection and body perception disturbances. No regions showed significantly greater activity in the sham illusion than the finger stretch.
In sum, this is the first study to identify the neural underpinning of a visuo-haptic illusion altering body perception.

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Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

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Emory University Research Committee

Title: Neural basis of the crossmodal correspondence between auditory pitch and visuospatial elevation

Authors: ***S. A. LACEY**[1], K. MCCORMICK[1], R. STILLA[1], L. NYGAARD[1], K. SATHIAN[1,2]; [1]Emory Univ., Atlanta, GA; [2]RR&D Ctr. for Visual and Neurocognitive Rehabilitation, Atlanta VAMC, Atlanta, GA

Abstract: Behavioral research has demonstrated systematic crossmodal correspondences for many perceptual domains, e.g., a high-pitched auditory stimulus is associated with a visual stimulus at a high spatial elevation, whereas a low-pitched auditory stimulus is associated with a visual stimulus at a low spatial elevation. The underlying neural mechanisms are unknown. In the present study, we used functional magnetic resonance imaging (fMRI) to examine the neural basis of the crossmodal correspondence between auditory pitch and visuospatial elevation. Participants (n=18) were presented with audiovisual stimuli in an event-related design, so as to be either congruent (e.g. a high-pitched tone and a circle positioned high in space) or incongruent with respect to the pitch-elevation correspondence. Participants engaged in a one-back (same/different) task. Reaction times (RTs) were recorded (RTc: RT for congruent trials; RTi: RT for incongruent trials). A neural congruency effect (CE), indexed by greater blood oxygenation level-dependent (BOLD) responses for congruent trials relative to incongruent trials, when each trial type was preceded by a trial of a similar type, was observed in: the right superior frontal gyrus, supplementary motor area (SMA), anterior insula, superior temporal sulcus (STS) and putamen; and bilaterally in the inferior frontal gyrus (IFG), postcentral gyrus (poCG), parietal operculum (POp), precuneus and parieto-occipital fissure. The precuneus and parieto-occipital CEs correlated negatively across individuals with the behavioral CE: (RTi - RTc)/(Rti + RTc); the negative correlation indicates that people with smaller behavioral CEs had stronger BOLD CEs. Many of the regions showing the BOLD CE are implicated in multisensory processing; the involvement of somatosensory cortical regions (poCG, POp) may reflect somatosensory correspondences of the stimuli. Separate localizers, run to test proposed explanations for crossmodal correspondences, established that the right STS, IFG and SMA were sensitive to audiovisual synchrony (the first two being preferentially active when audiovisual stimuli were asynchronous and the SMA when they were synchronous), while the right STS focus was also part of a region engaged during semantic processing. However, none of the regions showing the BOLD CE were active during magnitude estimation, arguing against this as a primary mechanism. Our findings suggest that the neural substrates for the pitch-elevation correspondence are spatially distributed and involve multisensory processes.

Disclosures: **S.A. Lacey:** None. **K. McCormick:** None. **R. Stilla:** None. **L. Nygaard:** None. **K. Sathian:** None.

Poster

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Title: Investigating the rubber hand illusion using electrocorticography

Authors: *A. GUTERSTAM[1], K. COLLINS[2], J. CRONIN[2], K. WEAVER[2], J. OJEMANN[2], H. EHRSSON[1];
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Abstract: The question of how the brain self-attributes body parts is a central issue in both cognitive neuroscience and applied neuroprosthetics. Studies using the rubber hand illusion (RHI), a perceptual illusion in which ownership of an artificial limb is elicited through the concurrent touching of a rubber hand in view and a participant's hidden real hand (Botvinick & Cohen 1998 Nature), have highlighted multisensory integration within peripersonal space as a key mechanism for limb self-attribution. Several neuroimaging studies have found an association between the experience of the illusion and increased fMRI-BOLD responses in multisensory brain regions in the premotor and posterior parietal cortices (e.g., Ehrsson et al. 2004 Science; Guterstam et al. 2013 JOCN). To date, however, no study has characterized the direct neurophysiological correlates of the RHI in humans. To this end, we studied the RHI using electrocorticography (ECoG).

Five patients with medically refractory focal epilepsy undergoing pre-surgical invasive seizure monitoring volunteered for this study. During the experiment, the subjects rested comfortably in their hospital bed with the head of the bed titled. Their arm was hidden behind a screen, while a lifelike rubber hand was placed in front of the screen in full view. To induce the illusion, a digital touch probe was used to repeatedly deliver touches to the rubber and real hand simultaneously. In two control conditions, the touches were applied asynchronously or using a rotated rubber hand, which are established methods to break the illusion.

The synchronous condition generated significantly higher ratings of rubber hand ownership than did the asynchronous and rotated control conditions (both $p<0.05$). The ECoG results showed that the synchronous illusion condition was associated with significantly ($p<0.05$, corrected) increased high-gamma power (70-150 Hz) in electrodes located along the intraparietal sulcus and the ventral and dorsal premotor cortex. In two patients, we conducted separate fMRI experiments prior to electrode implantation, allowing for a direct comparison between BOLD and electrophysiological responses within single subjects. In sum, these results constitute the first direct electrophysiological evidence that the feeling of ownership of a seen limb is associated

with increased neuronal activity in multisensory cortical regions. Finally, the superior temporal resolution of ECoG (1220 Hz) compared to fMRI (<1 Hz) renders it possible to study dynamic interactions between cortical regions of interest, potentially revealing important clues about the underlying neuronal mechanisms of bodily self-attribution.

Disclosures: A. Guterstam: None. **K. Collins:** None. **J. Cronin:** None. **K. Weaver:** None. **J. Ojemann:** None. **H. Ehrsson:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 532.19/AAA9

Topic: D.09. Multisensory Integration

Support: CUA Burns Fellowship

CUA Millennium Scholarship

Title: Practice order effects of arm movements with tactile vs. visual guidance

Authors: P. J. LEE, L. SIMPSON, *S. N. KUKKE;
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Abstract: Preliminary studies have provided evidence that performing touch-guided movement (TM) may promote improved performance on future visual-guided movement (VM). However, the specificity of this potential order effect to initial TM guidance remains to be tested. The overall goal of this study was to test the behavioral and electrophysiological effects of TM or VM practice on subsequent TM and VM tasks.
Twenty right-handed, healthy adults (19-34 yrs) entered this IRB-approved study. They traced 6 irregular shapes (path length=45.7±3.6 cm) with the right index finger using 1 of 2 sensory modalities for guidance. In the TM task, movement was guided by tactile feedback of bumps along the path. In the VM task, movement was guided by vision of dots along the path seen through a small circular window at the fingertip. Each participant performed 120 serial trials in one of four groups: 1) TM-VM (i.e., 60 TM followed by 60 VM trials, n=8); 2) VM-TM (n=8); 3) TM-TM (i.e., 120 TM trials, n=2); and 4) VM-VM, (n=2). The trace time of each trial and electroencephalographic (EEG) data from 28 scalp leads were recorded. Cortical activation was quantified by the drop in task-related power (TRP), the log-ratio of spectral power during the task to rest.
Trace time decreased with both TM and VM practice. Initial TM, as opposed to VM, practice

appeared to be associated with lower trace times in subsequent TM and VM trials. Cortical activation in the alpha (8-12 Hz) and beta (13-30 Hz) bands decreased to approximately zero during the VM task only when preceded by TM (and not VM) practice. There was a focal cortical activation pattern when TM was performed first, and a widespread pattern when VM was performed first. This initial sensorimotor set remained whether the subsequent task was the same or different from the first.
This study provides evidence that initial TM practice has the potential to influence the performance and neurophysiological state during future movements guided by touch or vision. After further data collection from individuals in the TM-TM and VM-VM groups, a full-factorial repeated measures ANOVA will be used to quantify effects of initial practice modality on trace time, alpha TRP, and beta TRP. Knowledge of the cross-modal transfer between TM and VM arm movements will form a scientific basis for the choice and practice order of therapeutic activities in neurorehabilitation of hand function.

Disclosures: **P.J. Lee:** None. **L. Simpson:** None. **S.N. Kukke:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

Location: Halls B-H

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Topic: D.09. Multisensory Integration

Support: Grants-in-Aid for Scientific Research, Japan

Title: Sound-contingent visual motion perception: Evidence from functional neuroimaging

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Abstract: After a paired presentation of visual apparent motion with the alternation of two different tones for several minutes, the tones are able to trigger visual motion perception for a static object (SCVM: sound-contingent visual motion). Here we investigated neural mechanisms underlying SCVM using an fMRI adaptation technique. In a 3-min association session, two white circles placed side by side were alternately presented. The onsets of the two circles were synchronized with high and low frequency tones, respectively. Before and after this association, test sessions were conducted while simultaneously measuring brain activity. A trial in each test session consisted of three successive conditions: apparent visual motion with the tones (3 s,

"AM1"), a blinking visual stimulus at a fixed location with the tones (6 s, "BL"), and AM2 (3 s), which is the same as AM1. The audiovisual stimuli of AM1 and AM2 were consistent with those of the association session. We expected that after the association was formed, participants would perceive visual motion in the BL as well as AM1 conditions, resulting in decreased activation in areas related to SCVM in the AM2 condition. Before the association, areas related to visual motion processing and to multisensory processing were activated in both the AM1 and AM2 conditions. In contrast, after the association, these areas were not significantly activated in the AM2 condition while continuing to be activated in the AM1 condition. These results suggest the involvement of visual motion and multisensory processing areas in SCVM. (1666 / 2300 characters)

Disclosures: W. Teramoto: None. **S. Higuchi:** None. **Y. Sugita:** None. **S. Hidaka:** None.

Poster

532. Multi-Sensory Integration: Crossmodal Processing in Humans I

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Topic: D.09. Multisensory Integration

Support: NIH grant R01EY012440

Title: Visual cortical activation during processing of shape metaphors in congenitally blind people

Authors: *V. OCCELLI[1], S. LACEY[2], R. STILLA[2], C. STEPHENS[2], K. MCCORMICK[3], D. KEMMERER[4], K. SATHIAN[2,5];
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Abstract: Numerous studies have shown that the visual cortex in congenitally blind people is more active than in normally sighted controls, during both language and sensory tasks. It remains uncertain whether the relevant non-visual functions reflect a combination of sensory and language processes, or whether one of these kinds of processing is dominant. It is possible that the visual cortex assumes non-visual sensory functions in those with congenital blindness, and that its recruitment during language tasks is due to the grounding of language in sensory processes. Metaphorical language is associated with sensorimotor cortical activity in areas processing domain-specific information relevant to the metaphors under consideration. For instance, metaphorical use of action verbs engages motor-related cortical activity (Desai et al.,

NeuroImage, 83: 862-869, 2013); textural metaphors recruit texture-selective somatosensory cortex (Lacey et al., Brain & Language 120: 416-421, 2012); and metaphors referring to body parts activate the extrastriate body area of visual cortex, an area responsive to visual images of body parts (Sathian et al., SfN Abstracts 204.1, 2014).
We therefore asked whether visual cortex in congenitally blind people is more responsive during processing of metaphors than in sighted people. This would be consistent with the hypothesis that sensory grounding of language drives visual cortical activation in congenital blindness. We tested this idea in the domain of shape using auditory presentation of sentences containing shape-related words in metaphorical and literal contexts, along with control sentences matched on a number of linguistic and acoustic variables, while congenitally blind and sighted control participants underwent functional magnetic resonance imaging (fMRI) in an event-related design. Analysis of the resulting fMRI data indicates that the congenitally blind group exhibited significantly greater metaphor-selective activity in multiple regions of visual cortex, compared to sighted controls. Some of these regions demonstrated shape-selectivity for visual stimuli in the sighted participants. These findings are consistent with the hypothesis that sensory grounding of language underlies visual cortical activation in congenital blindness, and offer a potential explanation for previous reports that visual cortex in congenitally blind people is responsive during both language and sensory tasks.

Disclosures: V. Occelli: None. **S. Lacey:** None. **R. Stilla:** None. **C. Stephens:** None. **K. McCormick:** None. **D. Kemmerer:** None. **K. Sathian:** None.

Poster

533. Reaching: Humans in Health and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 533.01/AAA12

Topic: E.04. Voluntary Movements

Support: FP7-PEOPLE- 2012-CIG- 334201 (REMAKE)

Title: Neural correlates and quantitative assessment of ankle joint position sense: a fMRI study.

Authors: *R. IANDOLO[1,2], A. BELLINI[3], G. BOMMARITO[3], I. MARRE[2], N. OESINGMANN[4], L. FLEYSHER[4], F. LEVRERO[3], G. MANCARDI[3], M. CASADIO[2,1], M. INGLESE[3,4];
[1]Italian Inst. of Technol., Genova, Italy; [2]Dibris, [3]DINOGMI, Univ. of Genoa, Genova, Italy; [4]Neurol., Mount Sinai school of medicine, New York, NY

Abstract: The ability to identify the position of lower limb joints in space is critical for ambulation, for maintaining balance and for any fine motor movements. The joint position sense is likely to be affected by neurological diseases since it results from a complex process of integration of sensory inputs from different peripheral receptors.
Despite its relevance, very little is known about the physiopathology underlying sensory deficits of distal lower limbs, especially in terms of neuronal correlates. Our final objective is to verify the hypothesis that, in addition to the motor dysfunction, deficits of lower limbs proprioception can induce specific neural modifications that impact daily life tasks such as standing and walking. As first step toward this goal, we investigate the ankle position sense in 23 healthy adults (12 female, 27.7 years, min 23 max 37). Proprioception was evaluated while performing the following unilateral and bilateral matching tasks with both the dominant and the non-dominant foot:
1.Unilateral matching: the ankle was passively moved toward a pre-defined inclination and back to the baseline position. Then, subjects were required to match the desired previous position with the same foot.
2.Bilateral matching: the subject's foot was moved to a predefined inclination. Subjects had to reach the same position with the contralateral foot. When the subjects reached the position, the ankles were repositioned back to the baseline position and another movement started towards a new inclination value. Four positions were presented: two for the plantar-flexion, one for the dorsi-flexion and one neutral. Each position was presented in pseudo-random order and at least four times. These tests were performed outside and inside the MRI environment with subjects lying in supine position. We used a custom built MRI-compatible device with two detached platforms that allow for the independent motion of each foot. The ankle movements were measured by two custom-made optical encoders. The protocol included also active and passive dorsi-plantarflexion motion of the dominant ankle and of both ankles (in phase). In addition to the fMRI, the MRI protocol includes structural T2 weighted and 3D T1 weighted sequences for brain volumetric assessment and Diffusion Tensor Imaging for microstructural assessment of white matter tracts. This protocol allowed us to characterize the ability to correctly identify the ankle position for each limb and to estimate the differences between the matching modalities as well as the dependence of the performance on the angular degrees of rotation.

Disclosures: **R. Iandolo:** None. **A. Bellini:** None. **G. Bommarito:** None. **I. Marre:** None. **N. Oesingmann:** None. **L. Fleysher:** None. **F. Levrero:** None. **G. Mancardi:** None. **M. Casadio:** None. **M. Inglese:** None.

Poster

533. Reaching: Humans in Health and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 533.02/AAA13

Topic: E.04. Voluntary Movements

Title: The characteristic of motor learning across lifespan

Authors: *M.-H. LEE;
Michigan State Univ., East Lansing, MI

Abstract: Do people at different ages learn motor tasks differently? One challenge is that motor learning ability in different age groups is also confounded by differences due to prior experience and changes in physical abilities such as speed and strength. Therefore, to minimize these differences, we utilized a virtual task which required participants to learn a novel upper body coordination pattern.
We examined the motor learning process at different ages (between 9 and 70 years of age) in a virtual reaching task. In this task, inertial measurement units were attached to the upper body and these movements were mapped to the position of a computer cursor. Critically, this map was customized so that each individual could complete the task using their own movement repertoire. The goal of the participants was to learn to move the cursor in a center-out reaching task to 8 different targets. There were 160 trials in total toward 4 targets during practice session. Three generalization tests were interleaved during learning (pre, during and post-practice), where participants reached toward all 8 targets. These tests were used to evaluate changes in performance with practice.
We found that in spite of using a novel virtual task to minimize usual confounds in studying age-related differences, there were clear age-related differences in learning. All groups improved with practice, but there was a U-shaped trend for movement time, with children (less than 12 years old) and older adults (more than 50 years old) taking significantly longer to perform the task than younger adults. Moreover, when we examined the rate of learning using an exponential fit, we found that the rates of learning also showed a similar U-shaped trend, with slower rates of learning in children and older adults compared to younger adults. These results show that age-related differences in motor learning cannot be explained simply as performance differences - instead, they reflect differences in underlying learning mechanisms.

Disclosures: M. Lee: None.

Poster

533. Reaching: Humans in Health and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: E.04. Voluntary Movements

Support: MARS Grant 90RE5010-01-01

Title: Building prediction models for clinical recovery after stroke

Authors: *Y. ABDEL MAJEED[1], S. AWADALLA[2], J. L. PATTON[1,3];
[1]Bioengineering, [2]Biostatistics, Univ. of Illinois At Chicago, Chicago, IL; [3]Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL

Abstract: Chronic stroke survivors respond to treatments in varying degrees, and prediction of recovery may help forecast recovery time and may even guide therapy by identifying important aspects of movement. Clinical evaluations allow some quantification of ability and function, but features captured by robotic devices allow a library of available metrics that could more accurately predict patient improvement. In this work, we use our knowledge on the first day of a study, including demographic information, to build first- and second-order predictive models that anticipate the patient's recovery over three weeks.

Twenty-six stroke survivors performed a reaching task in a 3D virtual environment. Subjects were matched through their intake Fugl-Meyer scores with scores ranging from 25 to 49 for Fugl-Meyer and from 2.59 to 49.24 for the Wolf Motor Function test. Extracted features of their movements, their demographic information, and their clinical ratings on the first day were used to predict outcomes at the end of our three-week randomized controlled clinical study. We employed two state-of-the art modeling techniques (LASSO regression and Random Forests) to obtain a robust model. This involved 100-fold cross validation and fitting each model using mean predictions.

Here, we present the results of our cross-validated predictive modeling efforts to accurately predict both clinical recovery (R^2 ranging from 33% to 99%, depending on the modeling method) and identify factors most relevant to the recovery process (speed-related metrics generally ranked higher, and were negatively correlated to clinical recovery). The best modeling method (Random Forests) predicted Fugl-Meyer at a level of 97% and Wolf Motor Function at 96%. This improves on previous predictions and also provides a ranking of the most important features. These features can then be targeted in future treatment designs to improve patients' performance and recovery time.

Disclosures: Y. Abdel Majeed: None. **S. Awadalla:** None. **J.L. Patton:** None.

Poster

533. Reaching: Humans in Health and Disease

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Title: Movement reorganization and space representation through the operation of a body-machine interface

Authors: *C. PIERELLA[1], F. ABDOLLAHI[2], E. THORP[2], A. FARSHCHIANSADEGH[3], I. SEANEZ[2], F. A. MUSSA-IVALDI[2], C. MAURA[4];
[1]Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL; [2]Northwestern Univ., Chicago, IL; [3]Northeastern Univ., Chicago, IL; [4]Univ. of Genoa, Genoa, Italy

Abstract: People with paralysis must reorganize their residual mobility for performing activities of daily living. We have developed a system, the body-machine interface (BMI) that facilitates this reorganization by capturing motions of the upper body through a set of inertial sensors and by mapping the sensor signals into the coordinates of a computer cursor. By moving the computer cursor, the BMI user can perform a variety of different tasks, such as controlling the direction and speed of a virtual or real wheelchair, entering text, playing computer games and navigating the internet. Importantly, as the users interact with the BMI they also perform physical exercises so structured to aim at specific rehabilitative goals, such as increasing the range of body motions, maintaining and improving strength, enhancing movement smoothness, coordination and symmetric control of right and left body sides.The BMI exploits the abundance of degrees of freedom, which even in severe forms of paralysis are in excess of the two coordinates necessary to specify the position of the computer cursor. Because of redundancy, the cursor can be placed at any target position by the user assuming one of many "equivalent" body configurations. Therefore, the BMI allows us to investigate the mechanisms underlying movement reorganization and gain a deeper understanding of motor learning and recovery. In particular, here we investigated the representation of the BMI formed by subjects while engaging in a variety of tasks. In principle, because of redundancy subjects may learn different ways to control the motion of the cursor, depending on the task that they are practicing. Alternatively, they may learn a single representation. We found that over an extended training period of 28

sessions, subjects tended toward similar representations. However, the similarity did not become an identity. As they engaged in different tasks, the subjects appeared to maintain a small but systematic residual difference in the body configurations used to place the cursor on the same locations. This is consistent with findings from other studies suggesting that people exploit redundancy to minimize task-related costs. The "null space" variability is not simply noise but it retains structure that reflecting control optimizations orthogonal to the kinematic goals of the task.

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Poster

533. Reaching: Humans in Health and Disease

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Topic: E.04. Voluntary Movements

Support: NICHD ROI HD072080

NIGMS 5R25GM79300

Title: Manipulating joint angle positions and visual feedback.

Authors: ***M. SMITH**;
Biomed. Engin., Northwestern Univ., Chicago, IL

Abstract: At the Rehabilitation Institute of Chicago Robotics lab, we investigate how the nervous system is capable of gathering extrinsic information about the environment and using this to change our motor control strategy by taking advantage of redundant degrees of freedom as we become more proficient at a motor task. In our experiment, we exposed subjects to a redundant system where they controlled the three angles of a 3-link planar arm-like mechanism, ultimately controlling the cursor located at the tip of the chain in the X-Y plane. This setup allows us to observe how subjects take advantage of the redundancy of the system. In fact, there exist multiple joint angles of the kinematic chain (Θ1, Θ2, and Θ3) to arrive at the same X-Y coordinate of the screen. Over a two day training period subjects were tasked with controlling the cursor on the end of the arm mechanism and place it on various targets. Subjects were divided into two groups: the first group had complete vision (CV) of the kinematic chain and the cursor; the second group, minimal vision (MV), could only see the cursor but not the arm mechanism. I expect that the information gained from this experiment will explain how we use visual

knowledge about the structure of a redundant mechanism for learning how to control it and to take advantage of the versatility offered by the abundance of its degrees of freedom.

Disclosures: M. Smith: None.

Poster

533. Reaching: Humans in Health and Disease

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Topic: E.04. Voluntary Movements

Support: NIH grant R01 HD072080

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Title: Designing movements by injecting visuomotor noise

Authors: *E. B. THORP[1,2], F. A. MUSSA-IVALDI[1,2,3];
[1]Rehabil. Inst. of Chicago, Chicago, IL; [2]Biomed. Engin., Northwestern Univ., Evanston, IL;
[3]Physiol., Northwestern Univ., Chicago, IL

Abstract: The human motor system is characterized by an excess in the degrees of freedom it must control compared to the number necessary to complete most tasks. Accordingly, every movement that we make is selected from a large set of seemingly equivalent movements. Some theories assume that the central nervous system (CNS) when offered the possibility to achieve a goal with different movements, selects the movement that is least effected by motor noise. These theories make the direct prediction that if the relationship between movements and noise changed, the movements people make would also change in a predictable manner. Here, we test the hypothesis that by strategically designing artificial signal dependent noise, we can elicit desired motor behaviors. We inserted noise into a novel motor task, where subjects used hand movements (4 degrees of freedom) to control a computer cursor (2 degrees of freedom). Measurements of bilateral hand movements were linearly mapped to the location of a cursor on a computer monitor. For this task, there are infinite hand postures that result in a single cursor location. Signal dependent noise was artificially inserted such that the jitter of the cursor at each location on the screen was dependent on the hand posture. We found that this paradigm is effective at biasing subjects towards learning to make movements that were less affected by noise. Subjects who trained with the noise paradigm learned to use coordination patterns that were significantly closer to the desired policy than subjects who trained with no additional noise. Interestingly, we also found that adding signal dependent noise led to significantly improved task

performance when visual feedback was removed. To further investigate this, we studied the relationship between exploration of the control space and bothtask performance and the ability to avoid task specific noise.These results confirm that people plan movements in an attempt to minimize task specific variability. Additionally, they validate the concept that designing signal dependent noise can be an effective means to elicit desired motor behaviors. These results have broad implications for designing more effective rehabilitation paradigms to improve the performance of human-machine interfaces.

Disclosures: E.B. Thorp: None. **F.A. Mussa-Ivaldi:** None.

Poster

533. Reaching: Humans in Health and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 533.07/AAA18

Topic: E.04. Voluntary Movements

Title: Intermittency frequency modulation produces changes in movement distributions along reach to target

Authors: *A. K. SHAH[1], J. L. PATTON[2];
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Abstract: Excessive movement variability, especially in individuals with neurotrauma (stroke, TBI), can cause people to exceed task limits and lead to falls or accidents in the workplace or other events of "high cost". This is especially true in novel task dynamics when a person must perform a new task such as playing guitar for the first time. People often have a multitude of actions to choose from when executing complex tasks. Action-selection in these tasks can produce variable outcomes as a result. Compounded with this action-selection variability is the variability that stems from movement execution, novelty of the task and distorted conditions from prior well-learned tasks. We had previously shown how a simple agent that stores negative experiences as local regions of cost can produce movement distributions similar to a task where subjects had to learn to distribute their motions within an invisible zero-cost region through a variant of the Multi-Armed bandit principle of explore and exploit, instead choosing to use the principle of explore and avoid. Here we show the same controller can be used to observe error variations in targeted reaching by modulating intermittency frequency of replanning and submovement generation and its effect on targeted reaching. Further development of this controller has been done to show the applicability of this paradigm on a multitude of tasks. It can

potentially be used as a predictive model of movement distributions given some chosen parameter values of replanning interval, motor noise, width of influence and penalty increment.

Disclosures: **A.K. Shah:** None. **J.L. Patton:** None.

Poster

533. Reaching: Humans in Health and Disease

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Program#/Poster#: 533.08/AAA19

Topic: E.04. Voluntary Movements

Support: Sparrow/MSU Center for Innovation and Research

Title: Modulating motor variability during bimanual reaching in stroke

Authors: ***R. RANGANATHAN**[1], R. GEBARA[2], M. T. ANDARY[2], J. R. SYLVAIN[2]; [1]Dept of Kinesiology, [2]Dept of Physical Med. and Rehabil., Michigan State Univ., East Lansing, MI

Abstract: There is controversy over the use of the "unaffected" hand in stroke rehabilitation - while certain therapies like constraint-induced movement therapy restrict the use of the unaffected hand, bimanual therapy encourages active use of both hands. In this study, we examined how the two hands compensate for each other during a bimanual reaching task that was redundant. Adults with chronic stroke, with mild to moderate arm impairments volunteered for the study. Markers were attached to joints on both arms to measure movement using a motion capture system. The goal of the participants was to perform a virtual reaching task - where they attempted to move a cursor on a screen to different targets by moving their hands on a table. Critically, the cursor position was "weighted" between the positions of the two hands - signifying that there were multiple solutions to perform the task. Participants completed the task in three weighting conditions with the weights on the affected hand and unaffected hand being - (i) 80%-20%, (ii) 50%-50%, and (iii) 20%-80%. Participants were not informed about which condition they were performing - they were simply asked to reach with both hands toward the targets on the screen as quickly as possible. We measured the trial-to-trial variability in the reaching direction and hand paths of both the affected and the unaffected hand. Results showed that even though participants were blinded to the varying weighted conditions, there was clear modulation of trial-to-trial variability depending on the weighting condition. Specifically, the hand that was weighted less (regardless of whether it was the affected or unaffected hand) showed higher variability, consistent with the idea of optimal feedback control, that only focuses on reducing deviations that affect the task goal. These results suggest that bimanual control of a

shared object may result in more disordered or "sloppy" control of the affected arm during practice, which may potentially affect recovery adversely.

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Poster

533. Reaching: Humans in Health and Disease

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Topic: E.04. Voluntary Movements

Support: W81XWH-12-JPC1-MPI-PSD

Title: Identification of surgical training level with data from clinical scenario simulations and sensorimotor tasks

Authors: *F. C. HUANG[1], F. A. MUSSA-IVALDI[1], C. M. PUGH[2], H. MOHAMADIPANAH[2]; [1]Sensory Motor Performance Prog, Rehabil. Inst. of Chicago, Chicago, IL; [2]Sch. of Med. and Publ. Hlth., Univ. of Wisconsin, Madison, WI

Abstract: Assessment of surgical skill has traditionally relied on expert observation and qualitative scoring. The goal of this study was to develop efficient, objective tools to identify surgical skill from recorded movement. Our unique study design provided performance metrics in sensorimotor tasks (reaching error, movement time, sensitivity to task changes), as well as gross motion features in clinical procedures (task completion time, working volume, smoothness, and maximum speed). We devised an interactive virtual environment in which participants could perform multiple testing modules, including movements within visual distortion, force perturbation, and a simulated tissue puncture task. In addition, our experimental apparatus featured physical stations that simulated essential clinical procedures, including laparoscopic ventral hernia (LVH) repair, bowel anastomosis, central venous catheterization, and bladder catheterization. Our current analysis focuses on how a combination of metrics can predict a surgeon's level of training. Our approach was to determine how features of movement might be associated with a defined subset of surgeons, and hence reflect the normative skill level of that population. We considered survey responses from surgery residents, including the number of years post-graduation (PGY, four levels), research years (RY, three levels), and clinical years (CY, three levels). We performed a multivariate analysis of variance (MANOVA) to determine a linear combination of metrics and transformation matrix that best separates the defined population groupings. Next, using data transformed according to MANOVA, we performed a

linear discriminant analysis with cross-validation (90% training, 10% testing) to relate the class type to the selected metrics. Our results showed successful classification using combined metrics from the sensorimotor tasks, and separately from the combined metrics from all clinical scenarios. The sensorimotor task model successfully identified surgery residents (n=47) for all three categories, with an accuracy of 41.2% for PGY, 55.2% for RY, and 61.0% for CY. Data from clinical scenarios successfully identified surgery residents (n=38), with an accuracy of 38.0% for PGY, 42.7% for RY, and 70.3% for CY. Our findings demonstrate that data acquired from surgical simulators can be used to identify the most likely training level of a surgeon. The superior identification of clinical years over other factors suggests clinical relevance of our selected simulator tasks. Our findings will aid evaluation of surgical performance, and target specific skill deficits in surgical trainees.

Disclosures: F.C. Huang: None. **F.A. Mussa-Ivaldi:** None. **C.M. Pugh:** None. **H. Mohamadipanah:** None.

Poster

533. Reaching: Humans in Health and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 533.10/AAA21

Topic: E.04. Voluntary Movements

Title: Coordinate representation in motor learning of object manipulation.

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Abstract: The selection of the frame of reference is a critical step in learning novel dynamics. There is an ongoing debate in sensorimotor learning about the frame of reference employed by the brain to represent novel dynamics. An early study (Shadmehr & Mussa-Ivaldi, 1994) asked this question by investigating generalization patterns after adaptation to velocity dependent force fields. It considered two distinct reference frames: extrinsic (endpoint based) and intrinsic (joint-level based) and made predictions of generalization patterns corresponding to each frame of reference when the arm configuration was changed. The results suggested that the dynamics was represented in intrinsic coordinates. These findings were reproduced in a number of following studies. However, other studies that focused on transfer of the learned dynamics from one limb to another or from bimanual learning to a single limb found evidence for extrinsic representations. All of these previous studies were focused on unconstrained movements of the hand in free space and when a force field disturbed these movements. In this study instead, we used object manipulation and asked participants to move the endpoint of a virtual planar double

pendulum. We have shown previously that in this task, participants started the experiment by moving along rectilinear paths. However, they eventually learned to move along the curvilinear paths that minimized the kinetic energy transferred to the transported object after they became familiar with the object dynamics. Here, the learning phase was followed by a generalization phase where the object was placed in a different orientation. In this scenario, if the dynamics are represented in an arm centered coordinate frame, the executed trajectories should remain unchanged during generalization trials. On the other hand, if the dynamics are represented in an object centered coordinate frame, the trajectories should change in order to follow the path of minimum energy corresponding to the new orientation and finally, the learned dynamics might not transfer at all to the generalization test. Our preliminary results indicate that participants revert to straight line trajectories after the object was placed in a different orientation suggesting that the learned dynamics did not transfer to a new orientation and therefore the dynamics were represented by a coordinate frame that was specific to the orientation of the object during the learning trials.

Disclosures: **A. Farshchiansadegh:** None. **F. Mussa-ivaldi:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant# R01EB019834

Undergraduate Research Opportunity Program Grant

University of Michigan Office of Research Seed Grant

Title: Learning new gait patterns: Age-related differences in skill acquisition and inter-limb transfer

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Abstract: Motor skills are important for performing many day-to-day activities and individuals need to learn, unlearn, and relearn a number these skills over the course of their lives. Many studies have shown that the ability to learn new manual skills declines with aging. Similarly, previous studies have demonstrated that older adults have impaired inter-limb transfer (i.e., the ability to transfer the learned skills to the opposite limb without actually practicing the task on

the opposite limb): although this finding is not consistent across studies. While the effects of ageing on motor skill learning and inter-limb transfer are well-studied in tasks that involve the upper limbs; less is known on tasks that involve the lower limbs. Further, the tasks used in these studies typically have less ecological validity, thereby limiting generalizability of the results to everyday activities. Here, we examined the effects of aging on motor learning, retention, and inter-limb transfer of a motor task involving the lower limb. The task involved learning of a new gait pattern while walking on a treadmill. Forty-five subjects (25 younger and 20 older adults) were tested on two consecutive days. On Day 1, subjects learned a new gait pattern by performing a target-matching task, where the target was an altered version of their regular gait. On Day 2, subjects repeated the task with their training leg to test for retention effects and then performed the target-matching task using their untrained leg (i.e., transfer leg). Trials without visual feedback were also collected on both days at the beginning and at the end of training. The changes in tracking error were computed to study the learning effects. The results indicate that training on Day 1 resulted in significant reduction of target-tracking error in both groups ($p<0.05$); however, tracking error was significantly higher in older adults at the end of training (i.e., reduced learning ability). Older adults also exhibited lower retention and inter-limb transfer in comparison with younger adults ($p<0.05$). Analysis of no vision trials indicated that older adults relied more on visual feedback in comparison with younger adults; however, this was only the case for the training leg. The differences in inter-limb transfer effects appear to be primarily due to lower learning and retention on the training leg, as both groups showed similar transfer effect when the differences in the training leg's error were accounted for. These results provide evidence supporting the decline in motor learning and inter-limb transfer of motor skills with ageing. Acknowledgments: NIH Grant# R01 EB019834, UROP Grant, UM Office of Research Seed Grant.

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Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant# R01 EB019834

Title: Self-powered robots to minimize motor slacking during rehabilitation

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Abstract: Robotic rehabilitation is a promising approach to recover lost functions after stroke or other neurological disorders; however, "motor slacking" - a phenomenon where the motor system reduces muscle activation levels and movement excursions to minimize metabolic and movement related costs - is a known limitation of externally driven robots. We hypothesize that motor slacking could be effectively minimized if individuals provide the power to move the limb via their own body power (e.g., from their opposite limb). To test this hypothesis, we developed a wearable body-powered exoskeleton device that mechanically couples the motions of the user's elbow joints. Seven healthy adults were tested on four conditions and surface electromyography (EMG) was used to quantify slacking throughout four testing conditions. The four conditions were: 1) active self-assist, where subjects wore the device on both arms and were instructed to use both to perform a target-matching task; 2) resting self-assist**,** subjects performed the matching task with their dominant arm while resting their non-dominant arm; 3) resting external-assist, subjects rested their non-dominant arm while the researcher wore the other side of the device and performed the matching task; and 4) active external-assist, where subjects were instructed to contract their non-dominant arm along with the researcher. The matching task required subjects to follow a sinusoidal trajectory by flexing and extending their elbow for 10 minutes under each condition. Muscle activation was recorded from the elbow flexor and extensors muscles of the driven arm. For analysis, we calculated the cycle-by-cycle normalized EMG amplitude (% maximum) over each period of the sinusoidal wave. We then calculated the average activation and change in activation (slope) over each condition. In line with our hypothesis, we found that subjects had higher levels of muscle activation during the conditions that required self-assist compared to the external-assist conditions. There was a significant difference ($p<0.05$) in the average activation between the resting self-assist and resting external-assist conditions for each muscle. Additionally, resting self-assist demonstrated the highest slope of all of the conditions tested. The results of this study show that the self-assist conditions minimized slacking, as subjects unknowingly activated their driven arm even when explicitly told not to do so and maintained their average activation throughout the condition. Further testing on subjects with hemiparesis is warranted.

Disclosures: E.P. Washabaugh: None. **E. Treadway:** None. **R.B. Gillespie:** None. **C.D. Remy:** None. **C. Krishnan:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: Texas Woman's University REP grant

Title: Learning a leg visuomotor rotation task in chronic stroke survivors

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Abstract: To study motor skill learning, previous studies utilize a visuomotor rotation task in which a large movement error is introduced by rotating the location of visual cursor relative to the actual hand path. Subjects therefore learned to gradually adapt their hand movements by rotating the hand path an equal amount in the opposite direction of the cursor rotation. Thus, movement errors can be reduced through trial-and-error practice. However, little is known regarding whether stroke survivors have deficits in adapting a leg visuomotor rotation task and also have deficits in recalling this newly learned leg task after a period of time, so called "savings". Growing evidence suggests that savings may be related to a process of consolidation in the motor cortex. Thus lesioning of the motor cortex may result in impaired savings of a visuomotor adaptation. To investigate the potential for adaptation and savings of a visuomotor leg task and whether it may be impaired by motor cortical damage, we induced visuomotor adaptation of leg reaching movements and tested subsequent savings in a small group of subjects with chronic stroke and hemiparesis and a small group of age-matched healthy adults. The task was a sitting planar leg reaching movement. Subjects were given visual feedback in real-time via a cursor display on a computer screen. One of four targets, equidistance from the start location at top-left, and top-right screen positions, was randomly presented per trial. To minimize visual online corrections, the real-time feedback of cursor motion was removed half way through each reach. During "Baseline", cursor motion and foot motion were matched. During "Adaptation", cursor motion was rotated 15 degrees counter-clockwise relative to the actual foot trajectory. "Savings" was tested by repeating the Adaptation condition at three time points (30 min, 24 hours, and 7 days) after the initial adaptation to the rotation. We calculated movement errors as the angular deviation of the foot path from a straight line path to the target at the time of peak velocity. We compared magnitudes of errors across Baseline, Adaptation and Savings conditions and across control and stroke groups. We hypothesized that both controls and individuals with chronic stroke and hemiparesis would be able to adapt leg reaching to the visuomotor rotation and that controls would show evidence of savings. Our preliminary results suggest that

adaptation and savings of a visuomotor leg movements does occur in healthy individuals, but are impaired in chronic stroke survivors. A better understanding of adaptation and savings of a leg task in chronic stroke survivors may help develop an effective gait intervention strategy.

Disclosures: S. Tseng: None. **S. Chang:** None.

Poster

533. Reaching: Humans in Health and Disease

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Topic: E.04. Voluntary Movements

Support: NIH Grant K01AG047926

NIH Grant R01AG045163

Title: Relationship between beta-amyloid deposition and motor ability, but not motor memory, in patients with amnestic Mild Cognitive Impairment

Authors: *S. Y. SCHAEFER[1], K. DUFF[2], J. M. HOFFMAN[2];
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Abstract: ^{18}F-flutemetamol is a novel radiotracer for imaging β-amyloid deposition that has shown potential for differentiating Alzheimer's disease (AD) from other neurodegenerative diseases. Although ^{18}F-flutemetamol uptake in the brain has been neuropathologically validated as a biomarker in AD and is associated with cognitive functioning in older adults, there have been no reports of ^{18}F-flutemetamol uptake related to motor functions, such as psychomotor speed, grip strength, or balance. Thus, the purpose of this study was to examine the relationship between ^{18}F-flutemetamol uptake and motor ability in non-demented older adults at risk for developing AD. Ten community-dwelling older adults diagnosed with amnestic Mild Cognitive Impairment were examined with ^{18}F-flutemetamol-PET imaging and an upper extremity motor task involving reaching, grasping, and object manipulation. Global composites of ^{18}F-flutemetamol standardized uptake value ratios in the cerebral cortex were normalized to the pons, with higher values indicating greater β-amyloid deposition. Motor ability was measured as the amount of time to complete the task, with higher values indicating poorer (slower) performance. All participants' dominant and nondominant hands were tested with the motor task. ^{18}F-flutemetamol uptake was positively correlated with motor ability for the dominant (r=0.72; p=0.017) and nondominant (r=0.55; p=0.10) hands. Because of the nondominant hand's poorer motor ability on the task relative to the dominant hand, participants were given nine additional

nondominant practice trials and were tested again one week later. ^{18}F-flutemetamol uptake was surprisingly unrelated to the nondominant hand's motor ability when re-tested (r=0.33; p=0.35), and had no association with either within-session (r=0.13; p=0.70) or one-week motor practice effects (r=0.17; p=0.64). These findings support the use of both motor and cognitive testing for identifying those at risk for cognitive decline, as both types seem to be related to β-amyloid deposition. These results also suggest potential rehabilitative approaches that could exploit intact learning systems in late life cognitive disorders.

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Poster

533. Reaching: Humans in Health and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 533.15/AAA26

Topic: E.04. Voluntary Movements

Title: Underlying contributors to visuomotor learning change with aging

Authors: *C. PERRY[1], T. M. HERTER[2], T. SINGH[2], A. HARRISON[2], H. BOYCE[2], K. GOINS[2];
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Abstract: Introduction: Many visuomotor skills, such as driving a car, are mediated by a combination of skilled limb movement and skilled visual search (voluntary eye movements used to actively gather visual information from the environment). We know that limb movement and visual search efficacy diminish with aging, but we do not know if their contributions to visuomotor learning also change with aging. Here we examine the extent to which visuomotor learning and its underlying processes change across adulthood. **Methods:** Eighteen young adults and eight older adults used an upper-limb robotic device with a virtual display to practice a bimanual visuomotor task one day a week for six weeks. In this Object Hit and Avoidance (OHA) task, 300 objects (8 geometric shapes) moved towards participants, who used virtual paddles representing each hand to hit away target objects (2 shapes, n=200) and avoid hitting distractor objects (6 shapes, n=100). Participants completed six trials of the OHA task each week for a total of 36 trials. Hand and eye kinematics were collected and used to compute measures of *Task Performance* (percent of targets hit), *Hand Movement Efficacy* (horizontal hand speed), *Visual Search Efficacy* (percent of objects pursued with the eyes), *Eye-Hand Coordination*

(distance between the eyes and hand at the time of target contact) and *Visual Recognition Speed* (mean distractor pursuit time). **Results**: Both groups showed similar improvements in task performance, though young adults exhibited better baseline task performance. Improvements in task performance in both groups were associated with improvements in visual search efficacy and eye-hand coordination. However, young adults (but not older adults) showed improvements in hand movement efficacy, whereas older adults (but not young adults) showed improvements in visual recognition speed. **Discussion:** Our findings suggest that, across adulthood, visuomotor learning is mediated by a combination of distinct and overlapping mechanisms. Improvements in visual search efficacy and eye-hand coordination contribute to visuomotor learning at all ages. However, motor learning (hand movement) is emphasized in young adults and perceptual learning (visual recognition speed) is emphasized in older adults.

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Poster

533. Reaching: Humans in Health and Disease

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 533.16/BBB1

Topic: E.04. Voluntary Movements

Support: Pilot Research Grant from the National Multiple Sclerosis Society

Title: Ataxia rehabilitation in multiple sclerosis through a targeted dance class

Authors: *C. LOPEZ-ORTIZ[1], A. M. SCHIEDLER[2], A. L. TISHA[2], D. L. KINNETT-HOPKINS[2], Y. C. LEARMONTH[2], R. MOTL[2];
[1]Kinesiology and Community Hlth., Univ. of Illinois At Urbana-Champaign, Urbana, IL; [2]Univ. of Illinois, Urbana-Champaign, IL

Abstract: Multiple sclerosis (MS) is a disease of the central nervous system with impaired neural signal transmission between the brain and the body. Walking impairment is present in MS, particularly with advanced disease. It has been noted that rehabilitation is the only way of improving function in MS. To our knowledge, there is a major gap in the existing evidence of rehabilitation and walking in MS. There is no documented evidence for improving smooth coordination of movements through exercise interventions among those with MS. We designed a targeted dance class (TDC) intervention based on classical ballet dance training for improving smoothness of movement in persons with MS who have clinical ataxia. All procedures were approved by the local IRB. Five participants with MS ages 49 to 65 participated in this study.

The participants had Expanded Disability Status Scale (EDSS) scores between 4.5 and 6.5. The clinical ataxia score on the International Cooperative Ataxia Rating Scale (ICARS) ranged between 5 and 28. The (TDC) was held twice per week for sixteen weeks with a winter pause of two weeks after the first eight weeks. During the winter break, the participants practiced at home with a TDC practice DVD. The class was taught by a researcher with classical ballet teacher certification and neurorehabilitation expertise. Paired t-tests of pre and post intervention values on the ICARS and smoothness of gait as quantified by the s-index (spectral arc length of velocity) of the top of the head marker in a 3 meter walk (Qualisys, Sweden) indicate statistically significant improvements on smoothness of movement and reduction on clinical ataxia scores ($p < 0.05$). We further investigated the partition and distribution of smoothness variability pre and post intervention across limb segments. The results give insight into the processes of motor learning and rehabilitation in MS through a whole body movement intervention in the form of a targeted classical ballet based dance class.

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Poster

533. Reaching: Humans in Health and Disease

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Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI Grant Number 16K12988

Title: A portable motor learning laboratory (pomlab)

Authors: *M. SHINYA[1], K. TAKIYAMA[2];
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Abstract: BACKGROUND AND AIM: Motor learning experiments have required a huge and expensive manipulandum in a laboratory. This makes it hard to collect behavioral data from many subjects and patients in clinical, sports and educational fields. To overcome these weak points, portable and easy-to-use experimental environment is required. METHODS: We have developed an application on Unity3D which runs on tablet devices that can measure motor learning behaviors (we refer the application as POrtable Motor learning LABoratory, or PoMLab). The application was developed using Unity 3D. In PoMLab, reaching movement to control a cursor in conventional experimental system with manipulandum was replaced with

tilting the tablet devices. Participants were instructed to bring the cursor to a target on the screen. Visuomotor adaptation was tested in a task where the motion of the cursor is rotated by a given angle with respect to the direction of actual tilt of the device. The relevance of our application was tested by a series of experiments in which 96 subjects participated. RESULTS: First, adaption to abruptly applied visuomotor rotation was confirmed in the PoMLab setup, and the results were explained by a state space model. Second, participants were able to adapt to gradually increasing visuomotor rotation without being aware of the perturbation. Then, the effect of screen size and vibration on the learning curve was tested using different tablet devices. We also compared the results obtained in our PoMLab setting and those obtained in conventional laboratory experiments using manipulandum. Notably, some participants were aware of the perturbation in the laboratory experiment, but no subject was aware of the perturbation in the PoMLab experiment. The estimated learning rate was comparable between the laboratory and PoMLab experiments, whereas the estimated retention rate tended to be lower for the PoMLab experiments than for the reaching experiments. CONCLUSIONS: We validated PoMLab application by demonstrating that it could reproduce the results obtained in conventional experiments involving reaching movements and visuomotor adaptation. The merits of PoMLab (i.e., we can conduct motor learning experiments at any time or place) would enables scientists, clinicians or teachers to measure motor learning performance from hundreds or thousands of subjects.

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Poster

533. Reaching: Humans in Health and Disease

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Program#/Poster#: 533.18/BBB3

Topic: E.04. Voluntary Movements

Support: CIHR MOP-125915

Title: White matter integrity and its relationship to cognitive-motor integration in females with post-concussion syndrome

Authors: ***J. HURTUBISE**, D. GORBET, C. HUGHES, A. MACPHERSON, L. SERGIO; Kinesiology & Hlth. Sci., York Univ., North York, ON, Canada

Abstract: Cognitive-motor integration (CMI) is required when performing movements where a rule is used to align the required motor output and the guiding visual information. We propose that measuring this integration under conditions which place demands on visual-spatial and

cognitive-motor processing may provide an effective behavioural means for detection of brain alterations associated with concussion. Our previous research has shown CMI declines in both university-aged[1] and child athletes[2] who had a history of concussion but were deemed recovered at the time of evaluation. The purpose of this study was to characterize the differences in symptoms, CMI, and white matter integrity (fractional anisotropy, FA) in those with post-concussion syndrome (PCS) versus healthy controls. Previously we have observed decreased white matter integrity in adults at risk for dementia related to their impaired CMI performance[3]. We hypothesize that there will be a similar decrease in white matter integrity in those suffering from PCS and that this will be correlated with performance declines on a CMI task. **Methods:** To date, 12 female participants are included in this study; 6 with post-concussion syndrome, and 6 age-matched healthy controls with no self-reported history of concussion. Participants were administered the current international sport concussion assessment tool (SCAT3), four visuomotor transformation tasks (3 of which required cognitive-motor integration), and diffusion weighted images were acquired. The visuomotor tasks were completed on a tablet linked to a desktop computer monitor. The participants displaced a cursor from a central target to one of four peripheral targets by sliding their finger on the tablet either directly to the viewed target or with decoupled eye-hand coordination (targets viewed on alternate plane, 180° cursor feedback rotation, or both). **Results:** We observed that those with PCS had worse symptom scores (both in number and severity) as well as impaired performance in CMI tasks. Those with PCS had decreased mean FA in bilateral corticospinal tracts beneath the premotor and primary motor cortices, and in the white matter underlying the right superior parietal lobule. **Conclusions:** Females with PCS had worse symptoms, decreased CMI performance, and decreased FA within the frontal-parietal-subcortical network. This decreased white matter integrity may be associated with both the CMI performance as well as symptom scores. Future work will investigate if CMI intervention may lead to better recovery for those suffering from PCS. **References:** [1]Brown et al. *BMC Sports Sci.* (2015) [2]Dalecki et al. *Conc.* (2016) [3]Hawkins et al. JAD (2015)

Disclosures: **J. Hurtubise:** None. **D. Gorbet:** None. **C. Hughes:** None. **A. Macpherson:** None. **L. Sergio:** None.

Poster

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Program#/Poster#: 533.19/BBB4

Topic: E.04. Voluntary Movements

Title: LEARNING: Lumbar excursion activities reinforced through novel interactive gaming

Authors: *M. E. APPLEGATE, S. T. LEITKAM, S. A. WALKOWSKI, C. R. FRANCE, J. S. THOMAS;
Ohio Univ., Athens, OH

Abstract: A fundamental clinical problem among individuals with chronic low back pain (LBP) is the avoidance of the very movements that are necessary for rehabilitation due to fear of injury or reinjury (kinesiophobia). The purpose of this study was to assess the effect of a short-term graded exposure virtual reality game intervention that encouraged lumbar movement on expectations of fear and harm and movement strategies during full body reaching tasks in adults with chronic LBP and kinesiophobia. During session 1, subjects were randomized into either the control or gameplay group; all subjects completed the standardized reaching task, which involved placing a physical ball on a shelf located at a position that elicited a theoretical 15, 30, and 60 degrees of lumbar flexion (with elbows fully extended and shoulders flexed to 90 degrees). On the following three consecutive days, the gameplay group played four levels of virtual reality dodgeball. Subjects wore 3D glasses and were oriented to their avatar on a 3DTV in the third person perspective. Virtual balls launched from virtual opponents to target locations on the avatar based on the reaching task excursions encouraged movement of the lumbar spine to successfully block and duck the balls. The game levels progressively became more difficult by lowering the projected target height of the launched balls. On gameplay days 2 and 3, target heights decreased by 5% and 10%, respectively. During session 5, all subjects repeated the standardized reaching tasks. During gameplay and reaching tasks, body movements were streamed from Vicon Tracker to The MotionMonitor, which recorded segment kinematics. A repeated measures ANOVA revealed a significant group (control, gameplay) by reaching task session (baseline, post-test) interaction on movement speed ($F = 5.913$, $p < 0.05$). Baseline movement speed was not different between the control group (1544 ± 77 ms) and the gameplay group (1425 ± 70 ms). Post-test movement speed in the control group (1530 ± 66 ms) was not different compared to baseline movement speed; however, post-test movement speed in the gameplay group (1280 ± 66 ms) was significantly faster than baseline movement speed ($p < 0.05$). There were no significant differences in degree of lumbar excursions between reaching task sessions. A series of one-way ANOVAs revealed that females in the gameplay group showed greater reductions in expected harm when reaching to the lowest target than women in the control group ($p < 0.05$). These findings suggest that our virtual reality gaming intervention has the potential to safely alter lumbar movement strategies and reduce expectations of harm in individuals with kinesiophobia and chronic LBP.

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Poster

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Topic: E.04. Voluntary Movements

Support: NSERC

Title: Rewarding task-relevant motor variability to optimize motor learning

Authors: *S. K. COLTMAN, L. E. BROWN;
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Abstract: It is unclear to what extent motor variability impedes performance when learning a new skill and to what extent it enables our ability to learn. Motor variability has been considered both noise that needs to be reduced to having a functional role in promoting motor learning. It has recently been shown using both a reward and error-based task that greater exploration along the task-relevant dimension of a task leads to faster learning rates during performance tasks (e.g. Wu et al., 2014). Given this finding, can performance variability be manipulated to improve learning rate? Can we task slow learners and make them learn more quickly by encouraging them (through reward) to explore the workspace more thoroughly? This study demonstrates that a participants' inherent tendency to produce what some might see as spatial errors can both predict their learning rate and be induced to improve learning. We designed an experiment to examine whether we could replicate the initial findings of this study and to assess whether levels of variability could be manipulated using a reward-based paradigm to enhance learning when adapting a simple visually guided reaching movement. Participants used their right hand to make simple point-to-point movements from a constant start position to one of four possible targets varying in direction, during baseline and visuomotor rotation tasks. Building on previous studies, we designed a reward-based feedback task intended to encourage exploration along the task-relevant dimension, specifically movement angle variability. We assessed whether we could induce task-relevant variability and whether this increase in variability would lead to an increase in learning rate during subsequent testing. Overall, we did not find a significant relationship between the amount of task-relevant variability during baseline and the initial learning rate across all participants. Additionally, we did not observe a significant increase in the portion of task-relevant variability or a significant increase in initial learning rate during subsequent testing of the visuomotor rotation task. Subsequent analyses will explore the hypothesis within individuals.

Disclosures: S.K. Coltman: None. **L.E. Brown:** None.

Poster

534. Functional Anatomy of Arm, Hand, and Finger Movement

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: E.04. Voluntary Movements

Support: NIH grants RO1 NS24328 (PLS)

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Title: Origin of descending commands from the cerebral cortex to hand motoneurons in the rat

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Abstract: The descending control of voluntary movements is mediated, in part, by cortical output neurons with relatively direct access to motoneurons. In the rat, the most direct connection between cortical output neurons and motoneurons is a disynaptic linkage (Yang and Lemon, Exp Brain Res, 2003). Here, we used retrograde, transneuronal transport of rabies virus (CVS-N2c strain, 45-50µl, $>10^8$ pfu) from a single muscle of Sprague-Dawley rats (n=3) to define the cortical regions that are the origin of disynaptic inputs to hand motoneurons. The muscle we injected was the right extensor digitorum communis (EDC). We set the survival time (~3.5 days) to enable retrograde transneuronal transport to 3rd order neurons. This enabled virus to move from the injected muscle to label the EDC motoneurons that innervate it (1st order neurons). Then, virus was transported transneuronally in the retrograde direction to label 2nd order neurons that are connected with EDC motoneurons (e.g. spinal interneurons). Finally, virus was again transported transneuronally in the retrograde direction to label 3rd order neurons in the cerebral cortex that are connected with spinal interneurons (e.g., corticospinal neurons). At this survival time we found that cortical neurons infected with rabies virus were strictly confined to layer V, the cortical layer known to contain corticospinal neurons. Most labeled neurons (> 95%) were located in the hemisphere contralateral to the injected muscle. Labeled neurons formed three distinct patches on the lateral surface of the hemisphere. The largest patch, accounting to 89-92% of the neurons labeled in the contralateral hemisphere, was located in the primary motor area (M1) and in the primary somatosensory area (S1). The second patch (7-10% of the contralateral labeled neurons) was located in the secondary motor area (M2) which is rostral and medial to M1. The third patch (~1% of the contralateral labeled neurons) was located in the secondary somatosensory area (S2) which is caudal and lateral to M1. These results indicate that the cortical control of forelimb movement in the rat originates from multiple cortical areas. Although the most substantial input originates from M1, parts of M2, S1 and S2 also are potential sources of descending commands.

Disclosures: **J. Rathelot:** None. **A. Nwankwo:** None. **P.L. Strick:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: Hartman Foundation

Title: Laminar organization and target specificity of thalamocortical inputs to primary motor cortex

Authors: ***O. K. SWANSON**, A. MAFFEI;
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Abstract: The thalamic nuclei for motor control receive converging signals from the cerebellum and basal ganglia and project their output to motor cortices. One of the major output targets of the motor thalamus is the primary motor cortex (M1), which integrates thalamocortical (TC) inputs with those from other cortical and subcortical motor areas, and sends signals down descending motor pathways to execute movement. Although the TC-M1 pathway has been well-characterized with tract tracing studies, the synaptic properties of this connection remain unclear. To identify the postsynaptic targets of the TC-M1 connection and determine the synaptic physiology of these inputs, we used optogenetic tools to selectively stimulate TC afferents while recording from M1 neurons in an acute slice preparation. Adult C57BL/6 mice of both sexes were unilaterally injected with AAV9-CAG-ChR2-Venus into the ventroanterior/ventrolateral (VA/VL) complex of the motor thalamus. Approximately 2 weeks later, brains were rapidly dissected and sectioned into acute slices containing the forelimb motor area of M1. Whole-cell recordings were obtained from M1 neurons and TC afferents were stimulated with trains of blue (470nm) light pulses at various frequencies. After recording, slices were processed to reveal morphology and laminar location of recorded neurons. Labeled TC afferents were found in all layers of M1, but were densest in Layer 2/3 (L2/3) and L5. Monosynaptic TC currents were recorded in a large proportion of M1 neurons in L2/3 and L5 (>90%). The amplitude of TC excitatory postsynaptic currents (TC-EPSCs) was similar across layers (L2/3 TC-EPSC=328.88±89.76 pA; L5 TC-EPSC=336.83±105.30 pA). Neurons in both layers showed paired-pulse depression in response to stimuli at 5Hz and 10Hz (L2/3-5Hz PPR=0.50±0.04, L2/3-10Hz PPR=0.44±0.04; L5-5Hz PPR=0.45±0.10, L5-10Hz PPR=0.46±0.10). Investigating the synaptic properties and postsynaptic targets of the connection between VA/VL and M1 is

instrumental for understanding of how cortical motor areas receive and integrate subcortical signals.

Disclosures: O.K. Swanson: None. **A. Maffei:** None.

Poster

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Title: The connectome for corticospinal outputs mediating distal forelimb control

Authors: *D. GIBBS, Y. TAKASHIMA, E. OVRUCHESKY, J. BIANE, M. H. TUSZYNSKI, J. M. CONNER;
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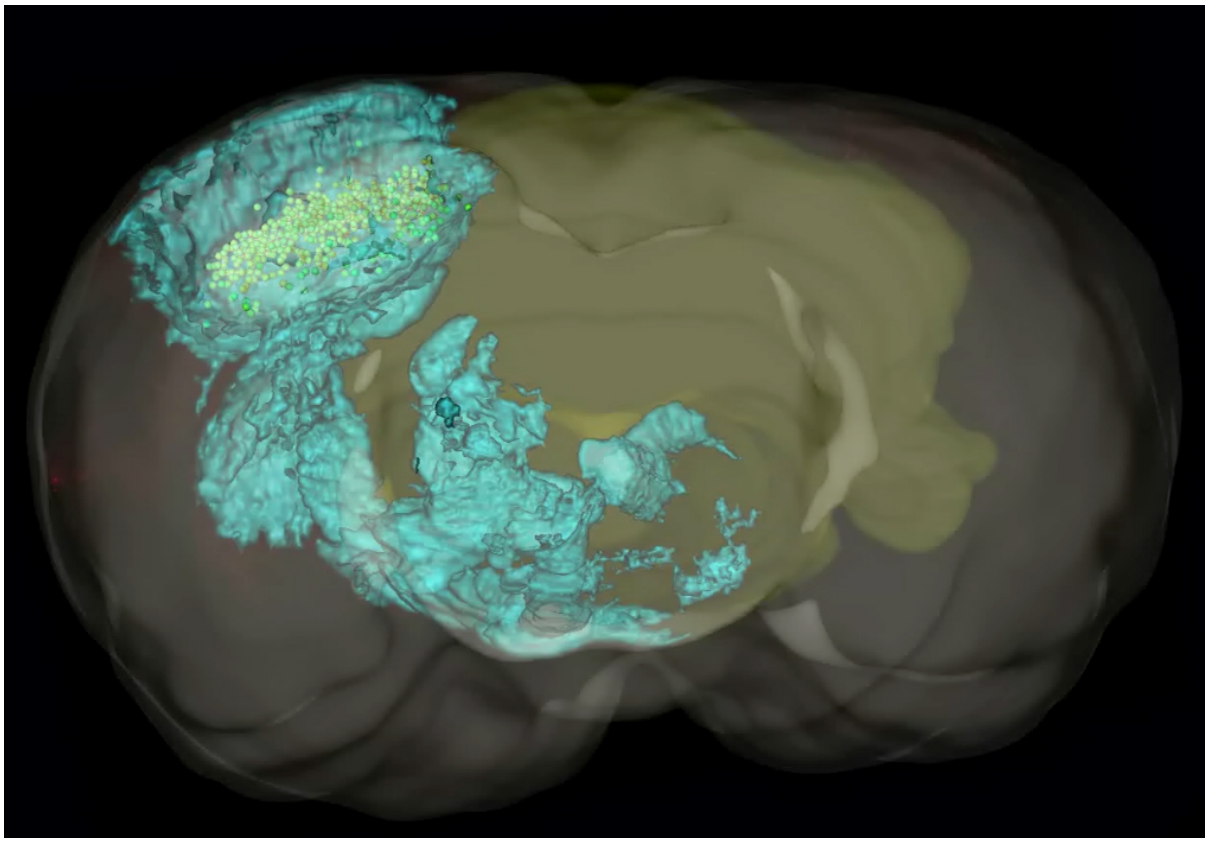
Abstract: Skilled motor behaviors engage a widely distributed network of motor and sensory processing. Primary motor cortex is a key hub in this network, playing roles in planning and orchestrating voluntary actions. While much of motor cortex action is likely mediated by direct projections to the spinal cord, efference copies of descending motor commands are distributed to other structures via collaterals from corticospinal projections. The extent of this collateralization is currently unknown. In the present investigation, a viral intersectional approach was adopted, using cre-dependent viral vectors and cre-expressing retrogradely transported rAAV vectors. rAAV vectors expressing genetically encoded tools, including optimized fluorescent tracers and optogenetic probes were targeted specifically to C8-projecting corticospinal upper motor neurons associated with distal forelimb control. The same intersectional approach was also taken to target cre dependent anterograde transsynaptic virus (HSV129 delta TK TT) to the same CST sub-population. Using three dimensional image reconstruction we visualized the spatial organization, axonal projection pattern and synaptic connectivity from a uniquely defined sub-population of

corticospinal upper motor neurons throughout the rat brain, brainstem and spinal cord. This approach allowed us to define the entire *connectome* specifically for corticospinal projections mediating distal forelimb control, and to functionally validate the postsynaptic targeting and connectivity of collaterals. Our findings reveal an unprecedented complexity in motor command signals arising from layer V of primary motor cortex, provoking a re-assessment of the role of motor cortex in mediating motor control.



Disclosures: **D. Gibbs:** None. **Y. Takashima:** None. **E. Ovruchesky:** None. **J. Biane:** None. **M.H. Tuszynski:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Acorda Therapeutics. **J.M. Conner:** None.

Poster

534. Functional Anatomy of Arm, Hand, and Finger Movement

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Topic: E.04. Voluntary Movements

Support: NIH Grant NS079471

Title: Columnar organization of primary motor cortex revealed with intrinsic signal optical imaging and intracortical microstimulation in squirrel monkeys

Authors: *O. A. GHARBAWIE, A. R. SLOAN;
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Abstract: The hand representation within primary motor cortex (M1) is central to manual dexterity. Neurophysiological studies of the M1 hand representation have shown that its intrinsic organization lacks a distinctive topographic pattern. In addition, anatomical studies have shown dense interconnections linking most of the M1 hand representation and that it lacks architectonic compartmentalization. Consequently, the columnar organization that characterizes most primary cortical areas are in question for the M1 hand representation. The goal of the present study was to examine the organization of the functional connections of sites within the M1 hand representation. We used an approach that allowed to study the connectivity of individual microelectrode sites and could therefore reveal organizational features that may have been obscured with traditional anatomical tracings. In two squirrel monkeys, we used intracortical microstimulation to map the locations of M1 sites from which digit movements were evoked. To investigate the functional connections of those sites, we delivered trains of pulses (150 pulses, 0.2 ms/pulse, 300 Hz) to the approximate location of layers II/III. We imaged the resulting changes in the intrinsic signal under 630 nm or 540 nm wavelength illumination. The most prominent activation domain (~1 mm^2) was invariably within the immediate vicinity of the stimulating microelectrode. In addition, 1-3 activation domains (200 - 400 μm^2) overlapped the M1 hand representation. The spatial locations of the activation domains shifted with site of microstimulation. The anatomical connections linking two domains within M1 were driven with equal efficacy from either domain. For comparison purposes, we mapped the functional connections of select sites within the hand representations in somatosensory areas 3b and 1. Intra-areal activation for each of those sites was limited to a single domain in the immediate vicinity of the stimulating microelectrode. Inter-areal domains of activation overlapped hand representations that matched the representations identified at the microstimulation site. Our somatosensory results confirm that our approach faithfully reveals cortical connectivity. Thus, the focal domains that we identified within the M1 hand representation are likely a reflection of its columnar organization.

Disclosures: O.A. Gharbawie: None. **A.R. Sloan:** None.

Poster

534. Functional Anatomy of Arm, Hand, and Finger Movement

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Title: Neural synergies in the primary motor cortex without muscle synergies

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Abstract: High-dimensional neural or muscular spaces can be reduced mathematically to low-dimensional synergies that capture most of the original variance. Such synergies can decrease the computational burden of controlling multidimensional outputs. However, the link between neural and muscle synergies remains uncertain. If muscle synergies are driven by neural synergies in the primary motor cortex (M1), muscle synergies and M1 neural synergies should share similar properties and activation patterns when used to reconstruct the original muscular and neural recordings.

Three monkeys (*Macaca mulatta*) performed a task in which they reached to, grasped, and manipulated 4 objects each in up to 8 locations. We recorded electromyographic (EMG) activity in arm and hand muscles, and spiking activity from microelectrode arrays implanted in caudal M1. Non-negative matrix factorization (NNMF) was applied to extract time-varying (spatiotemporal) muscle synergies, and time-varying neural synergies from simultaneously recorded activity.

Previous studies of time varying synergies have selected the number of synergies based on a sharp bend (genu) in the curve of reconstruction R^2 as a function of the number of synergies, all of a single fixed duration. We extracted different numbers of synergies, N (1 to 10), of different durations, T (10 to 610 ms), from muscle activity and from neural activity separately. We then examined plots of reconstruction R^2 as a function of N and T for a genu indicating an innate number of synergies. Whereas for muscle synergies none of the 3 monkeys showed such a genu, for neural synergies all 3 monkeys showed a genu—at N=3 synergies in two monkeys, and at N=2 synergies in the third. In each monkey the corresponding muscle synergies did not follow these neural synergies either in order or in amplitude of activation.

A limited number of muscle synergies have been identified in other studies that involved a limited variety of upper extremity movements. We suggest that the lack of innate muscle synergies in our data reflect the greater variety of upper extremity movements in the present study. Although we have no clear explanation for the presence of a small number of neural

synergies, our findings fail to support the hypothesis that M1 neural synergies drive muscle synergies in generating reach, grasp, and manipulate movements.

Disclosures: Z. Liu: None. **A.G. Rouse:** None. **M.H. Schieber:** None.

Poster

534. Functional Anatomy of Arm, Hand, and Finger Movement

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Title: Modeling the subcellular distribution of synaptic connections in cortical microcircuits

Authors: *S. DURA-BERNAL[1], B. A. SUTER[2], S. A. NEYMOTIN[3], G. M. G. SHEPHERD[4]; [1]Physiol. and Pharmacol., State Univ. of New York Downstate Med. Ctr., Brooklyn, NY; [2]Inst. of Sci. and Technol. Austria, Vienna, Austria; [3]Physiol. & Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY; [4]Physiol., Northwestern Univ., Chicago, IL

Abstract: Understanding cortical microcircuits requires not only mapping connections at the level of cell populations, but also determining the location of synapses along dendritic trees. Experimental evidence has highlighted very specific patterns of subcellular organization that depend on the brain region, cell type and cortical depth. These distinct patterns of innervation are likely to subserve important neural coding functions and have effects at multiple spatiotemporal scales, including the meso- and macro-levels. Incorporating accurate subcellular connectivity in cortical computational models is however challenging. Experimental data is generally sparse and obtained using a variety of techniques and formats, and neuronal simulators tend to lack specific tools to ease the task of synapse placement. Hence, we developed NetPyNE, a Python package to facilitate the development of biological neuronal networks in the NEURON simulator, with an emphasis on the incorporation of multiscale anatomical and physiological data. NetPyNE seamlessly converts a set of a simple, standardized high-level specifications in a declarative format, into a NEURON model. The subcellular distribution of synapses along the dendrites can be specified, and is automatically adapted to the morphology of each model neuron, which could range from a few to thousands of compartments. Different methods are available to provide subcellular synaptic location information, including 2D density maps derived from subcellular

Channelrhodopsin-2-Assisted Circuit Mapping (sCRACM) data; 1D cortical-depth maps; or using subsets of dendritic trees (section lists) based on the more common categorical data found in the literature (eg. layer 4 cells targeting the layer 1 apical tuft). Using this tool we were able to analyse the network-level effects of synaptic distribution in a multiscale model of primary motor cortex (M1) with morphologically detailed layer 5 corticospinal and corticostriatal cells. Our work demonstrates the important role of subcellular patterns in investigating cortical microcircuits, and facilitates future collaborations between anatomists, physiologists and modelers.

Disclosures: S. Dura-Bernal: None. **B.A. Suter:** None. **S.A. Neymotin:** None. **G.M.G. Shepherd:** None.

Poster

534. Functional Anatomy of Arm, Hand, and Finger Movement

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Topic: E.04. Voluntary Movements

Title: Changes in intracortical inhibition due to an external focus of attention

Authors: *W. TAUBE, Y.-A. KUHN, J. RUFFIEUX, M. KELLER;
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Abstract: Introduction: Although it is well established that an external focus of attention (EF) contrasted to an internal (IF) or neutral focus of attention enhances motor performance and motor learning (Wulf, 2012), little is known about the neural mechanisms underlying these behavioral improvements. This study therefore aimed to i) clarify whether the focus of attention (EF vs. IF) influences motor performance when executing identical fatiguing tasks of the first dorsal interosseus (FDI) muscle and ii) outline differences in neural activity of the primary motor cortex (M1). For this purpose, subthreshold transcranial magnetic stimulation (subTMS) was used as this technique is known to inhibit ongoing motor cortical output without affecting spinal structures (Davey et al., 1994; Lazzaro et al., 1998). Thus, differences in subTMS-induced EMG suppression between an EF and an IF indicate distinct activity in M1. Methods: In session 1, 14 subjects performed an isometric finger abduction at 30% of maximal voluntary contraction (MVC) until task failure with either an IF or EF. In session 2, the same task was performed with the other focus. In session 3, subTMS was applied to the contralateral M1 to compare the activity of M1 during EF and IF of the same task. Results: When subjects performed the biomechanically identical tasks with an EF, the time to task failure was significantly prolonged compared to an execution with an IF (t_{13} = -2.73, p = 0.01). Analysis of subjects' RPE showed a significant main

effect of time with $RPE_{initial}$ being significantly lower than RPE_{final} ($F_{1, 60} = 160.33$, $p < 0.001$, $\omega^2 = 0.99$). However, there was no difference between conditions (IF vs. EF, $F_{1, 60} = 0.021$, $p = 0.88$, $\omega^2 = 0.0001$). In addition, there was no significant interaction effect (time × condition, $F_{1, 60} = 0.186$, $p = 0.66$, $\omega^2 = 0.001$). Subthreshold TMS resulted in a significantly larger suppression of EMG activity in the EF condition compared to the IF condition (t_9=-4.32, $p = 0.001$). Discussion: Our data shed new light on the neural mechanisms underlying attentional foci by showing that the activity of inhibitory circuits within M1 is modulated differentially by the type of attentional focus. The increased inhibition with EF may contribute to the more efficient movement execution with this kind of focus. In this respect, our results support the constrained action hypothesis suggesting a more targeted and thus, less widespread motor activation with an EF (Wulf et al., 2001).

Disclosures: W. Taube: None. **Y. Kuhn:** None. **J. Ruffieux:** None. **M. Keller:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NSERC

CIHR

Title: Changes in cortico-spinal excitability associated with motor learning by observing

Authors: *H. R. MCGREGOR[1], V. C. RINCHON[1], P. L. GRIBBLE[1], R. CHEN[2], M. VESIA[2]; [1]The Univ. of Western Ontario, London, ON, Canada; [2]Krembil Res. Inst., Toronto Western Hosp., Toronto, ON, Canada

Abstract: While many of our motor skills are acquired through physical practice, we can also learn how to make movements by observing others. For example, individuals can learn how to reach in novel dynamical environments (‘force fields’, FF) by observing the movements of a tutor. Previous repetitive transcranial magnetic stimulation (rTMS) and fMRI studies suggest a role for the motor system in motor learning by observing. Here, we hypothesized that changes in cortico-spinal excitability support motor learning by observing. To test this idea, we used single-pulse TMS to assess changes in cortico-spinal excitability at rest following the observation of a FF learning task. Healthy participants grasped the handle of a InMotion2 robotic manipulandum with the right hand and were instructed to perform straight reaches to visual targets in a

horizontal plane. In a baseline condition, participants performed reaches in a null field, in which the robot applied no force to the hand. Before each intervention, we assessed the offline excitability of primary motor cortex (M1) and cortico-spinal networks by using single-pulse TMS to elicit motor evoked potentials (MEPs), measured at the right first dorsal interosseous (FDI) and the right abductor pollicis brevis (APB). Participants were then randomly assigned to one of two intervention groups: a learning group (n=15) and a control group (n=15). The learning group observed a video of a tutor adapting his reaches to a clockwise FF. The control group observed a video of a tutor performing reaches in an unlearnable FF, in which the direction of the force varied randomly from trial-to-trial. Immediately after each observational intervention, we repeated the neurophysiological measures and also assessed motor learning behaviorally by asking subjects to reach to targets in the presence of a counterclockwise FF (the opposite FF to what had been learned in the video). Motor learning by observing was thus assessed in terms of proactive interference in the counterclockwise FF. For the learning group, motor learning by observing was accompanied by increases in MEP amplitudes in right FDI and right APB. In contrast, control participants who observed the tutor performing reaches in the unlearnable FF did not exhibit changes in subsequent MEP amplitudes. This suggests that motor learning by observing involves functional changes in M1 or cortico-spinal networks, or both.

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Poster

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Title: Effect of baclofen on coupling between hand and forearm muscles after spinal cord injury

Authors: *J. LONG[1], T. TAZOE[1], D. S. SOTEROPOULOS[2], J. C. ROTHWELL[3], M. A. PEREZ[1];
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Abstract: Although baclofen is a $GABA_B$ receptor agonist commonly used to relief spasticity after spinal cord injury (SCI) its effects on voluntary motor output remain poorly understood. We previously demonstrated that corticospinal excitability in an intrinsic finger muscle, tested during a precision grip task, changed to a different extent in SCI subjects that take or do not take baclofen (Bunday et al., 2014). Because multiple hand and forearm muscles contribute to the control of a precision grip between the thumb and index finger, we investigated the effects of baclofen on the ability to couple hand and forearm muscles during the same task. We examined intermuscular coherence between hand [first dorsal interosseous (FDI), abductor pollicis brevis (APB)] and forearm [extensor carpi radialis (ECR), flexor carpi radialis (FCR)] muscles in the alpha (8-15 Hz) and beta (15-30 Hz) frequency band during index finger abduction and a precision grip in participants with and without incomplete chronic cervical SCI. We found that coherence between FDI-FCR and FDI-ECR in the alpha and beta frequency band was reduced during precision grip compared with index finger abduction in controls but remained unchanged in SCI participants. Notably, long-term (~4.6 years) use of baclofen after SCI decreased FDI-FCR coherence during a precision grip to similar levels as present in controls. To further examine the mechanisms involved in these effects, we measured the duration of cortical silent period (CSP; likely involving $GABA_B$ mediated mechanisms) in the FDI muscle across tasks. CSP duration decreased during precision grip compared with index finger abduction in controls and in SCI subjects taking baclofen and FDI-FCR coherence in the beta band correlated with the changes in the duration of CSP. Thus, our results suggest that GABAb-ergic inter-neuronal pathways may contribute to interactions between hand and forearm muscles during a precision grip task.

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Poster

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Craig H. Neilsen Foundation Grant 261299

Title: Distinct cortco-cortical contributions to human precision and power grip

Authors: *P. FEDERICO, M. A. PEREZ;
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Abstract: The corticospinal tract contributes to the control of finger muscles during precision and power grip. The involvement of different sets of cortical interneuronal circuits during these distinct grasping behaviors remains unknown. To examine this in humans noninvasively we used transcranial magnetic stimulation (TMS) over the hand representation of the primary motor cortex to elicit motor evoked potentials (MEPs) in an intrinsic finger muscle during index finger abduction (control task), precision grip, and power grip. The TMS coil was oriented to induce currents in the brain in the latero-medial (LM), posterior-anterior (PA), and anterior-posterior (AP) direction to preferentially activate corticospinal axons directly and early and late synaptic inputs to corticospinal neurons, respectively. We found that AP-LM MEP latency differences were consistently longer during power grip compared with index finger abduction and precision grip, while PA-LM differences remained similar across tasks. A paired-pulse induced MEP-peak, targeting AP but not PA inputs, increased during power grip compared with other tasks. Our novel findings suggest that cortical structures activated by PA and AP stimuli are differentially active during precision and power grip. We propose that a preferential recruitment of late synaptic inputs to corticospinal neurons may be achieved when humans perform a power grip.

Disclosures: P. Federico: None. **M.A. Perez:** None.

Poster

534. Functional Anatomy of Arm, Hand, and Finger Movement

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R01 AR-050520

USC Viterbi School of Engineering Doctoral Capstone Fellowship

Title: Control of a human cadaveric hand via autonomous extraction of high-dimensional joint probabilities of command signals

Authors: *B. A. COHN[1,2], K. JALALEDDINI[3], S. CHAKRAVARTHI[4], F. J. VALERO-CUEVAS[3];
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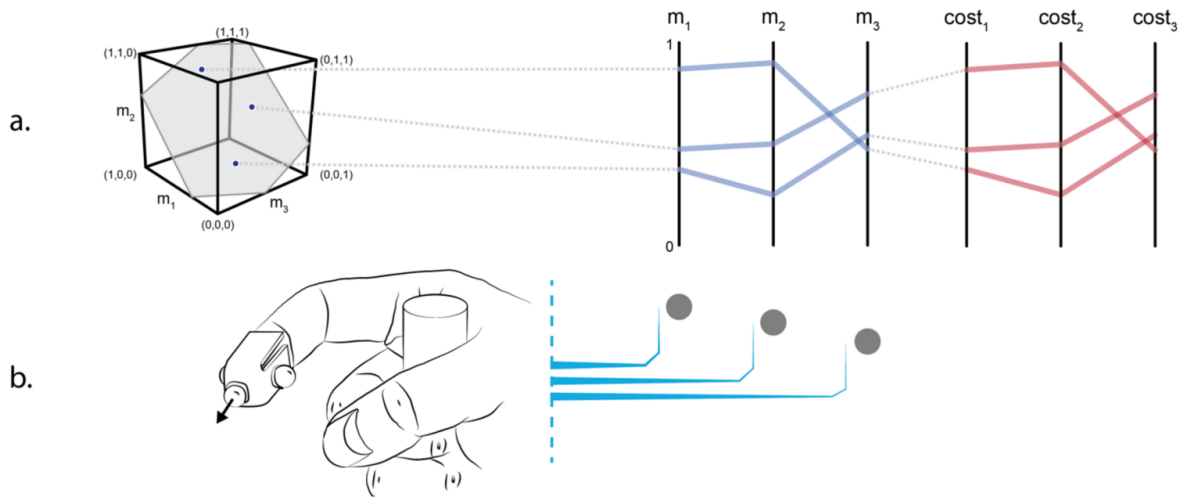
Abstract: Motor control is the quintessential Big Data problem. How does the nervous system identify and select a particular family of command signals given the high-dimensional set of potential options? While the motor control field has emphasized optimization and cost functions, we have proposed an alternative approach where the family of all feasible muscle coordination patterns has a strong structure defined by the mechanical structure of the limb and the functional constraints defining the task. Historically, finding the structure of such subspaces has been prohibitively expensive from the computational perspective given the high-dimensionality of the problem (e.g., to produce a fingertip force, one must find the 4-dimensional feasible set embedded in 7-dimensional spaces). We describe our use of the Hit-and-Run algorithm to describe these subspaces via their joint probability functions and present a high-performance computational cluster capable of robotically controlling a human cadaveric hand to autonomously extract these joint probabilities. This methodological breakthrough now allows us to explore the way in which motor learning is coupled to motor performance when faced with a high-dimensional control problem. In particular, we emphasize that this trial-and-error, memory-based, and Bayesian-like approach is in fact technically feasible, and may be more biologically plausible for neuromuscular systems than optimization strategies driven by cost functions and gradients. This fault-tolerant approach to motor control also has important implications to motor-relearning in the context of rehabilitation, because our autonomous algorithm can continue operating and learning from the finger, even after properties of the limb have been altered. To inform modern prosthetic control algorithms & the way muscles are controlled in healthy and disease states, this research details a viable and usable big-data approach to a recursive learning algorithm implemented on a human cadaveric finger.



Disclosures: **B.A. Cohn:** None. **K. Jalaleddini:** None. **S. Chakravarthi:** None. **F.J. Valero-Cuevas:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 535.01/BBB17

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NS080586

NS086372

Title: Dissecting V1 interneuron function within the mammalian locomotor network

Authors: ***S. DI COSTANZO**[1,2], G. GATTO[2], M. GOULDING[2];
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Abstract: The locomotor networks commonly referred to as the locomotor central pattern generator (CPG) comprise of motor neurons and five “core” interneuron (IN) populations designated as V0, V1, V2a, V2b and V3 INs. Our lab has shown that the V1 INs are important for regulating the speed of locomotion and play an essential role in the control of flexor-extensor movement. The aim of my work is to understand how V1 INs are integrated within the locomotor system by using mouse genetics in combination with viral tools to undertake a

morphological and connectivity analysis. Our strategy to analyze V1 morphology is to fill neurons using an innovative approach based on EnvA pseudotyped G-deleted rabies tagging of V1 INs in a Cre-dependent manner. In this way we can identify discrete subpopulations/cell types based on shared morphological features and correlate this with existing transcriptional profiles. To better understand how V1 INs are integrated within the spinal cord network, we also are mapping the distribution of the V1 IN presynaptic partners. This second approach is based on a tripartite intersectional genetic system that utilizes the combined activities of Cre and Flp recombinase to selectively express the EnvA receptor and the rabies G protein in V1 INs located in the caudal spinal cord. Our ultimate goal is to obtain better insights into the function of V1 INs by unveiling its anatomical organization.

Disclosures: S. Di Costanzo: None. **G. Gatto:** None. **M. Goulding:** None.

Poster

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Title: Activity of individual spinal neurons during forward and backward locomotion

Authors: P. V. ZELENIN[1], P. E. MUSIENKO[2,3], O. V. GORSKII[2], V. F. LYALKA[1], N. MERKULYEVA[2,3], Y. P. GERASIMENKO[2], G. N. ORLOVSKY[1], *T. DELIAGINA[1]; [1]Karolinska Inst., Stockholm, Sweden; [2]Pavlov Inst. of Physiol., St. Petersburg, Russian Federation; [3]Inst. of Translational Biomedicine, St. Petersburg State Univ., St. Petersburg, Russian Federation

Abstract: Higher vertebrates including humans are capable not only of forward locomotion but also of walking in different directions relative to the body axis [backward (BW), sideward, etc.],

as well as of stepping in place. While the neural mechanisms responsible for the control of forward (FW) locomotion were studied in considerable detail, the mechanisms controlling steps in other directions are mostly unknown. Recently we have shown that, when locomotor mechanisms of the spinal cord, brainstem, and cerebellum are activated by spinal cord stimulation, the direction of stepping is determined by sensory information from the limb signalling the direction of limb movement during stance. In the absence of this information, in-place stepping is observed. These findings suggest that the locomotor system includes two principal mechanisms, one generating a vertical component of step (limb elevation and lowering), and the other generating a horizontal component (limb transfer from one extreme point to the other). The aim of the present study was to reveal spinal neurons contributing to generation of the vertical and horizontal components of step during locomotion. For this purpose, in decerebrate cats activity of spinal interneurons from L4-L6 was recorded (by means of micro-arrays with 32 recording sites) during both FW and BW locomotion caused by epidural stimulation of the spinal cord at L5-L6. By using spike-sorting procedure, activity of individual neurons was extracted from the mass activity. 25% of neurons were modulated during neither FW nor BW locomotion, suggesting that they do not contribute to their generation. 75% of neurons were modulated during FW and/or BW locomotion. According to their activity during FW and BW locomotion, these neurons were divided into three groups. Group 1 contained neurons (33%), which had the same phase of modulation (maximal activity, burst position) during both FW and BW locomotion. We suggest that Group 1 neurons belong to the network generating the vertical component of steps (elevation and lowering the limb) during locomotion. Group 2 contained neurons, which were modulated during FW locomotion only (10%) or during BW locomotion only (10%). We suggest that Group 2 neurons belong to networks controlling direction of stepping (FW and BW, respectively). Finally, Group 3 neurons (22%) changed the phase of their modulation in locomotor cycle depending on the direction of locomotion. One can suggest that Group 3 neurons control muscles of the hip joint. At this joint, direction of the movements in swing and stance phase are opposite during FW and BW locomotion. Group 1-3 neurons were intermixed in the grey matter.

Disclosures: **P.V. Zelenin:** None. **P.E. Musienko:** None. **O.V. Gorskii:** None. **V.F. Lyalka:** None. **N. Merkulyeva:** None. **Y.P. Gerasimenko:** None. **G.N. Orlovsky:** None. **T. Deliagina:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: Onassis Foundation

Title: Cervical propriospinal neurons regulate locomotion based on sensory input derived from spatiotemporal position of the animal

Authors: *S. K. KARADIMAS[1], K. SATKUNENDRARAJAH[2], S. GOSGNACH[3], M. G. FEHLINGS[2];
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Abstract: Reticulospinal input is critical for the activation of the locomotor Central Pattern Generators (CPGs). In addition to this pathway long descending cervical propriospinal neurons projecting to ventromedial regions of lumbar spinal cord are required for well-coordinated locomotion. Although, most hold the view that the propriospinal neurons play a coupling role between forelimbs and hindlimbs during quadrupedal locomotion, there is some evidence that propriospinal neurons originating in the cervicothoracic region are involved in activation of the CPG. Currently, very little is known about the identity, anatomical distribution and the specific connectivity of these neurons to components of the locomotor CPG. Most importantly, a direct functional link between these neurons and locomotor activity has thus far has not been described. First, to illuminate the identity of cervical neurons with direct synaptic connections to the rhythmogenic area of the locomotor CPG, we utilized an intersectional technology by crossing *Vglut2::cre* or *VGAT::cre* mice with *Ai65(RCFL-tdT)* line that contains FRT-stop-FRT and LoxP-stop-LoxP double cassettes ahead of tdTomato. Unilateral injections of the retrograde *CAV-FLEx^{loxP}-Flp* were targeted to the L1-2 spinal segments of these double transgenic *Vglut2::cre; Ai65(RCFL-tdT)* or *VGAT::cre; Ai65(RCFL-tdT)* mice. This strategy reliably demonstrated that cervical neurons projecting to lumbar are glutamatergic. To further understand the role of lumbar-projecting cervical neurons in motor control, we aimed to determine the identity of presynaptic populations regulating these neurons. We applied retrograde monosynaptic labeling selectively initiated from the lumbar-projecting cervical neurons. We found majority of the direct input onto lumbar-projecting cervical neurons arose from the forelimb and hindlimb areas of the somatosensory cortex. Moreover, a strong population of presynaptic cells was also detected in the medial vestibular nucleus of the brain stem. Studies have showed that the somatosensory cortical area receives real time information regarding limb position and the locomotor speed during walking. Finally, we found that both selective ablation or silencing of the lumbar projecting cervical neurons resulted in progressively decreased speed and cadence during overground locomotion compared to controls. This phenotype was also characterized by a significant increase in the inactive time and decrease in the mobile time compared to control mice indicative of a perturbed ability to initiate and maintain mobility.

Disclosures: S.K. Karadimas: None. **K. Satkunendrarajah:** None. **S. Gosgnach:** None. **M.G. Fehlings:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: CIHR MOP-110950

CIHR MOP-136981

Title: Synaptic connectivity patterns of spinal V3 interneurons

Authors: *J. W. CHOPEK, Y. ZHANG;
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Abstract: V3 interneurons (INs) are excitatory, commissural INs that regulate robust, coordinated walking. Previous work from our lab utilizing dextran for synaptic tracing has demonstrated that V3 INs almost exclusively project contralateral in both the ascending and descending direction. However, dextran labelling may not be ideal to investigate local connections due to tissue damage and fluorescence saturation near the injection site. Therefore we used the Phasor spatial light modulator (SLM) system to investigate potential local V3 synaptic connections. This was achieved by exciting presynaptic neurons by uncaging MNI-glutamate over the soma with the Phasor SLM system while whole-cell patch clamp recordings were made in post-synaptic visually identified V3 INs (tdTomato fluorescence) or motoneurons (retrograde labelling) in 300 um thick slices from the lumbar spinal cord of $Sim1^{Cre/+;Rosafloxstop26TdTOM}$ mice. Using this method, we demonstrate V3 INs make local connections with ipsilateral motoneurons as well as with adjacent ipsilateral ventral V3 INs. Based on the latency from stimulation to the onset of the EPSPs recorded in V3 INs, these local V3-V3 connections are mediated at least in part by electrical coupling. The use of uncaging MNI-glutamate via the Phasor SLM system was also utilized to examine descending V3 IN connections in the horizontal slice preparation in which the ventral horn of the lumbar spinal cord remains intact. Using this horizontal slice preparation, we demonstrate that descending V3 INs synapse on contralateral motoneurons. Typically a cluster of adjacent V3 INs would need to be stimulated to elicit post synaptic potentials (PSPs) in contralateral motoneurons. These PSPs were generally excitatory although inhibitory PSPs were also noted on occasion. In addition to the different PSPs recorded, variable latencies from stimulation to the onset of the PSPs recorded in motoneurons were also found, suggesting that V3 INs have both mono- and polysynaptic connections with contralateral motoneurons. Our findings confirm previous results that demonstrate V3 INs are commissural interneurons and further our understanding of their connectivity by demonstrating local connections between adjacent V3 INs and between V3 INs and ipsilateral MNs.

Disclosures: **J.W. Chopek:** None. **Y. Zhang:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

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Support: NIH grant R01 NS081713

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Title: Spinal circuits controlling speed-dependent gait expression in quadrupeds: insights from computational modeling

Authors: ***S. M. DANNER**[1], S. D. WILSHIN[2], C. BELLARDITA[3], N. A. SHEVTSOVA[1], O. KIEHN[3], I. A. RYBAK[1];
[1]Dept. for Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Structure and Motion Laboratory, The Royal Vet. Col., Univ. of London, London, United Kingdom; [3]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: With increasing speed of locomotion, most quadrupeds, including mice, demonstrate sequential gait transitions from walk to trot, and to gallop and bound. Neural mechanisms underlying these transitions are poorly understood. We suggest that the speed-dependent gait expression results from speed-dependent changes in interactions between spinal circuits controlling different limbs and inter-limb coordination. As a result, the expression of each gait depends on (1) left-right interactions within the spinal cord mediated by different commissural interneurons (CINs), (2) fore-hind interactions on each side, and (3) supra-spinal drives to rhythm-generating circuits and CIN pathways. We developed a computational model of spinal circuits consisting of four rhythm generators (RGs; one for each limb). In this model, left-right interactions between RGs were mediated by V0 CINs ($V0_D$ and $V0_V$ sub-types) promoting left-right alternation and conditional V3 CINs promoting left-right synchronization. Fore and hind RGs on each side mutually inhibited each other. Using this model, we demonstrated that increasing external ("brainstem") excitatory drives to the RGs and V3 CINs could produce a progressive increase in the locomotor speed accompanied by sequential changes of the gait from walk to trot and to gallop and bound. The increase of locomotor speed resulted mostly from shortening the extension phase. Walk was expressed at low frequencies when the extension

phase was substantially longer than the flexion phase and transitioned to trot with the convergence of the extension and flexion phase durations. The transition from alternating (walk and trot) to synchronous gaits (gallop and bound) occurred when the activity of drive-receiving V3 CINs promoting left-right synchronization overcame the activity of V0 CIN supporting left-right alternation. The model closely reproduces and suggests explanations for the speed-dependent gait expression observed in vivo in intact mice and in mutants lacking $V0_V$ or all V0 CINs. Specifically, expression of trot is lost after removal of $V0_V$ CINs, and only bound is expressed after removal of all V0 CINs. The speed-dependent gait transitions, the existence of multi-stable regimes (coexistence of different gaits at the same speed), and the role of different neuron types, including CINs, and supra-spinal drives are analyzed with predictions for future experimental studies. The model provides important insights into the organization of spinal circuits and neural control of locomotion.

Disclosures: **S.M. Danner:** None. **S.D. Wilshin:** None. **C. Bellardita:** None. **N.A. Shevtsova:** None. **O. Kiehn:** None. **I.A. Rybak:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

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NIH Grant G12RR03051

Title: Identification and characterization of thoracic neural circuits in the mammalian spinal cord

Authors: E. CABEZAS-BOU[1], *M. E. DIAZ-RIOS[2,3];
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Abstract: The control of our trunk-related muscles is essential in order to coordinate locomotion in limbed and limbless animals. Limbless animals are able to move via a longitudinal coordinated wave of muscle contractions combined with segmental alternating contractions (half-center neural circuit organization). With the evolution of limbs, trunk neural networks had to now interact with these new limb-related networks and with sensory feedback in order to produce fluid movements. Studies regarding the control of movement in vertebrates have been mostly focused on limb-related neural networks. But the organization of trunk-related neural

networks have been largely unexplored. Thus, fundamental questions remain unanswered: Have the trunk neural networks of limbed vertebrates preserved elements of their segmentally-organized limbless ancestors? How is the trunk-related neural circuitry of limbed mammals organized? And, how does this network coordinates motor activity with or without limb-related networks? Studies have shown that the lumbar network entrains the thoracic network during locomotion suggesting a passive role of this trunk-related circuitry. We hypothesize that the trunk-related circuitry can have a principal role during posture and locomotion. Our results show that the thoracic cord coordinates with the lumbar cord during locomotor-like activity displaying a motor output with similar temporal dynamics (parameters). More interestingly, the isolated thoracic spinal cord can produce synchronous or alternating patterns of motor activity independent of lumbar (limb-related) neural networks and the motor output displays much slower temporal dynamics suggesting postural/balance-related control of movement. Alternating activity was elicited in the presence of a high-divalent solution suggesting that these rhythmic output was mostly coordinated through monosynaptic connections. Moreover, the use of blockers for inhibitory neurotransmission (strychnine and picrotoxin) disrupted this rhythmic alternating pattern. These findings support our overarching hypothesis that trunk-related motor output is at least partly produced by a half-center circuit organization which is likely evolutionarily conserved from limbless vertebrates. Further experiments will be directed toward identifying the organization of the trunk-related neural network, and the role of sensory feedback in thoracic motor control.

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Poster

535. Rhythmic Motor Patterns: Connectivity

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NIH Grant HL126522

A. P. Giannini Fellowship

Title: Mapping projections of excitatory and inhibitory preBötzinger Complex neurons

Authors: ***C. F. YANG**, J. L. FELDMAN;
Neurobio., UCLA David Geffen Sch. of Med., Los Angeles, CA

Abstract: Understanding the neural control of breathing requires a detailed neuroanatomical and functional map of the breathing central pattern generator (CPG). The logical entry point in constructing such a map is the "engine" of respiratory rhythm, the preBötzinger Complex (preBötC), a bilateral microcircuit of ~3000 interconnected neurons in the ventrolateral medulla. The preBötC generates respiratory rhythm in rodents (and presumably all mammals, including humans); rhythmic activity in the preBötC is required to produce inspiratory movements. To delineate the breathing CPG circuit in mice, we used a viral strategy to identify projections from molecularly-defined populations of preBötC neurons. We located efferent projections emanating from two broad, functionally distinct classes of neurons: excitatory neurons that express somatostatin (SST) and inhibitory neurons that express the glycine transporter GlyT2, using stereotaxic delivery of Cre-dependent AAV expressing EGFP (AAV-flex-EGFP) into the preBötC of SST-Cre or GlyT2-Cre mice. Critically, we optimized the viral delivery method to restrict viral transfections within the preBötC, while sufficiently labeling neurons to visualize long distance projections, enabling reliable identification of preBötC projections. Glutamatergic SST+ preBötC neurons projected to brainstem nuclei implicated in the control of breathing including the contralateral preBötC, parahypoglossal/nucleus of the solitary tract, ventral respiratory group, and parabrachial/Kölliker-Fuse nuclei, similar to efferent projections in rats; these neurons also had suprapontine projections to multiple discrete nuclei within the periaquaductal grey, thalamus, and hypothalamus, suggesting that the preBötC relays respiratory information to higher brain regions that may modulate physiological or behavioral processes. Strikingly, glycinergic preBötC neurons send projections in parallel with glutamatergic SST+ neurons to the same brainstem and suprapontine targets.

Disclosures: **C.F. Yang:** None. **J.L. Feldman:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: Howard Hughes Medical Institute

Title: A wiring diagram of central complex in *Drosophila* larva

Authors: ***A. FUSHIKI**, A. CARDONA;
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Abstract: The central complex (CX) is an anatomically recognizable structure in the insect brain. Functional studies indicate that the CX has a role in navigation and motor control, integrating inputs from multiple sensory modalities. A detailed wiring diagram with synaptic resolution remains to be elucidated. Here, we show for the first time neurons and circuits of the CX with synaptic resolution, reconstructed from serial section electron microscopy in Drosophila larva. The anatomical description of identified neurons in the CX of the adult fly led us to identify similar neurons in the larval brain. In the larval CX, we found neurons that traverse the columns of the protocerebral bridge, others that traverse the columns of the fan-shaped body, and ring neurons that define the ellipsoid body, albeit all these structures are split across the midline into two halves. We also found vertical neurons whose dendrites collect inputs from individual columns of the protocerebral bridge and fan-shaped body, and whose axons traverse the ellipsoid body and innervate a region ventrally to it--presumably the noduli. In prior work, we reconstructed the complete mushroom body (MB) and all neurons postsynaptic to the MB output neurons. We will detail both the CX circuitry and the connection between the MB and the CX, which suggests that learning can alter the motor functions associated with the CX.

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Poster

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Support: American Heart Association Grant

Title: Testing the limits of bipedal walking coordination: interlimb coupling in extremely asymmetric conditions

Authors: *K. FJELD, D. TAN, E. VASUDEVAN;
Hlth. and Rehabil. Sci., Stony Brook Unversity, Stony Brook, NY

Abstract: Legged animals frequently adjust spatial and temporal aspects of gait in order to walk, run, gallop, or turn. However, animals in nature rarely modify the stepping ratio between their two sides (e.g., taking two steps on one side for each step on the other). Although asymmetric stepping ratios tend not to occur under normal conditions, they can be induced using a split-belt treadmill that drives one leg faster than the other in cats [1] and human infants [2], suggesting that there is some degree of autonomy in the pattern generators controlling each leg's movement. In the many studies of human adults walking on split-belt treadmills, however, there have been

no reports of asymmetric stepping ratios. This may be because adults have not been challenged with a great enough speed differential between the belts for this behavior to emerge - previous experiments have only tested up to a 4-fold differential. Alternatively, the evolution and maturation of bipedal walking may have strengthened the coupling between the two legs, restricting the ability to produce asymmetric stepping ratios. Our objective was to investigate the limits of lower limb coordination in human adults. Ten adults (age 20-31 years) with no history of neurologic or orthopedic conditions were placed in a body weight support (BWS) harness suspended over a split-belt treadmill. They walked on split-belts running at a 10-fold speed differential (0.5:5.0m/s) in three different conditions: (1) no BWS, (2) 50% BWS, and (3) 50% BWS with explicit instruction to perform a 2:1 stepping pattern. In the first two conditions, subjects were told to walk without any specific instructions. Spontaneous 2:1 steps were scarce in the two conditions without instruction: there were no occurrences of 2:1 steps in the 50% BWS condition, and only one 2:1 step occurred in one subject in the no BWS condition. Subjects varied in their ability to produce 2:1 steps when explicitly told to do so. On average 2:1 steps made up 57% of the total steps taken in the instruction condition, but this ranged between 4% (low incidence of 2:1 steps) and 100% (perfect 2:1 stepping) for individual subjects. This suggests that, while adults show some ability to modify coordination voluntarily, asymmetric stepping ratios do not emerge automatically when the two legs are driven at very different speeds. A lifetime of bipedal walking experience may have caused pattern generators to become highly interdependent, possibly reflecting a strengthening of commissural interneuronal pathways or increased voluntary control over walking.
Forssberg et al. (1980).Acta Physiol Scand, 108:283-295.
Yang et al. (2004). Can J. Pharmacol. 82: 662-674.

Disclosures: **K. Fjeld:** None. **D. Tan:** None. **E. Vasudevan:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

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Support: CIHR Grant 15129

NSERC Grant 217435

GLFC Grant 54021

DAAD

GSfBS

UzK

Title: A stop signal for swimming originates from the mesencephalic locomotor region

Authors: *S. GRÄTSCH[1,2,3], F. AUCLAIR[2], D. VEILLEUX[2], A. BÜSCHGES[3], R. DUBUC[1,2]; [1]Groupe de Recherche en Activité Physique Adaptée, Univ. du Québec à Montréal, Montréal, QC, Canada; [2]Groupe de Recherche sur le Système Nerveux Central, Univ. de Montréal, Montréal, QC, Canada; [3]Zoologisches Inst., Univ. zu Köln, Köln, Germany

Abstract: In vertebrates, the mesencephalic locomotor region (MLR) plays a crucial role in controlling locomotor activity. Via a descending pathway it activates reticulospinal (RS) cells in the brainstem, which in turn project to central pattern generators (CPGs) in the spinal cord. The neural mechanisms underlying the initiation and maintenance of locomotion are partly understood, whereas the termination mechanisms are far more elusive. In the lamprey, distinct RS cell populations were found to control locomotor initiation ("Start Cells"), maintenance ("Maintain Cells"), and termination ("Stop Cells") [Grätsch et al., 2015; SfN 421.20]. During swimming, "Stop Cells" were found to generate a burst of spikes right before the end of locomotion (termination burst). Pharmacological activation and inactivation experiments demonstrated that "Stop Cells" play a crucial role in locomotor termination. We have shown that membrane properties are unlikely involved in generating the stop signal, suggesting the presence of specific synaptic inputs from other brain regions which still need to be identified. Here, we investigate the origin of the stop signal and show that the MLR is a very likely candidate. We performed experiments in the lamprey semi-intact preparation, in which swimming movements of the intact body can be recorded and correlated to the cellular activity of brainstem RS neurons. In this preparation, electrical stimulation of the MLR initiates locomotion that often outlasts MLR stimulation (28.02 ± 14.18 s; n=5). During the swimming period exceeding the MLR stimulation, we found that a stimulation pulse delivered in the MLR at a lower intensity (50 % of control) halted locomotion (within 6.83 ± 3.09 s; n=5). On the other hand, a stimulation pulse delivered at a higher intensity (100 % of control) prolonged the swimming bout. The MLR had similar effects on spontaneous or sensory-evoked locomotion. Local ejection of D-glutamate in the MLR lead to the same results: a small quantity of D-glutamate terminated ongoing swimming (within 8.41 ± 4.34 s; n=3), whereas a large quantity prolonged it. When "Stop Cells" were recorded intracellularly a termination burst was seen as locomotion was stopped by MLR stimulation. "Maintain Cells", however, repolarized in this situation. We propose that the MLR provides a synaptic input to "Stop Cells", which is the source of the termination burst. Funded by CIHR, NSERC and GLFC. SG received studentships from DAAD, UzK.

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Poster

535. Rhythmic Motor Patterns: Connectivity

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Title: Coordination of irregular bursting rhythms in central pattern generators

Authors: *I. ELICES[1], D. ARROYO[1], R. LEVI[1,2], F. B. RODRIGUEZ[1], P. VARONA[1];
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Abstract: The motor function of Central pattern generators (CPGs) circuits relies in the production of robust yet flexible rhythms in the form of regular spiking-bursting activity in each of their member neurons. Thus, both theoretical and experimental studies on CPGs typically consider regular spiking-bursting regimes.

In this study, we aim to understand the fast rhythm negotiation of CPG neurons by means of experimental and theoretical analyses of the pyloric CPG activity in situations where irregular yet coordinated rhythms are produced. In particular, we focus our study in the context of two sources of rhythm irregularity: intrinsic damage in the preparation, and irregularity induced by ethanol. Our departing hypothesis is that the analysis of non-periodic regimes can unveil important properties of the robust dynamics controlling rhythm coordination in this system.

Membrane potential was recorded from the LP and PD neurons, which form a half-center oscillator in the pyloric CPG of *Carcinus maenas*. The level of irregularity and coordination of the neurons' activity were analyzed using measures characterizing the cells' instantaneous waveform, period, duty cycle, hyperpolarization, temporal structure of the spiking activity, and measures describing instantaneous phases among them in the irregular rhythms and their variability.

Different sources of irregularity unveil distinct aspects of the flexibility and coordination of the CPG rhythm, particularly affecting the burst and hyperpolarization waveforms and their durations. Our results illustrate the strong robustness of the circuit to keep LP/PD anti-phase relationships under all conditions analyzed. In spite of being electrically coupled to the pacemaker cell of the circuit, the PD neurons showed a wider flexibility to participate with longer burst durations and larger increase in variability in the CPG rhythm. A conductance-based model of a half-center oscillator was used to assess the role of asymmetry to shape the irregular but coordinated activity observed experimentally.

The experimental and modeling results from the analysis of irregular CPG rhythms presented in this work illustrate that the rich dynamics of neurons and connections in the pyloric circuit

contribute to balance flexibility and coordination to readily negotiate their rhythms. We also discuss the presence of preserved relationships in the non-periodic but coordinated bursting activity of the pyloric CPG, and their role in the fast rhythm negotiating properties of this circuit.

Disclosures: I. Elices: None. **D. Arroyo:** None. **R. Levi:** None. **F.B. Rodriguez:** None. **P. Varona:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 535.12/CCC2

Topic: E.07. Rhythmic Motor Pattern Generation

Title: Temperature-sensitive and temperature-stable modules in spinal locomotor networks activity

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Abstract: Adult homeotherms experience reduced motor performance and fatigue from body temperature changes due to external conditions, fever, torpor or hibernation. In contrast, perinatal mammals display motor activity in a larger range of body temperatures, with rhythm enhanced at low temperatures in some species while suppressed in others. To understand how spinal networks produce these phenomena we studied temperature effects on motor activity. The perinatal murine in vitro spinal cord, when exposed to neuro-active drugs or afferent fiber stimulation, elicits ventral root activity with rhythm and pattern that resemble locomotion. Stimulation of descending fibers in this preparation elicits locomotor-like activity with flexor-extensor and left-right alternating pattern at a frequency of about 0.5 Hz at 23-24^{o}, and the frequency increases to about 1 Hz at 27^{o} C (n=4). Higher temperatures did not further accelerate the rhythm, but tended to disrupt activity. Bath application of NMDA and 5HT produces locomotor activity whose frequencies vary from 0.2 Hz to 1.6 Hz at temperatures 21^{o} to 35^{o} C (n=9). Plotting frequency as function of temperature showed a linear increase in frequency with a Q_{10} of 2.59±1.04 (n=9). Within this range, locomotor-activity showed highly conserved flexor-extensor and left-right alternation (n=9). Increasing temperature from 31^{o} to 35^{o} C resulted either in further increase in the frequency (50 %) or collapse of locomotor-activity (50 % crashes) with disruption of the flexor/extensor and left/right alternating pattern, or severe frequency reduction. Analysis of locomotor activity showed that burst amplitude displays an inverse U-shaped curve in response to temperature with the highest amplitude at 27^{o} C (n=9). Raising the Ringer's flow

from 14 to 20 ml/min succeeded in consistently improving locomotor-activity and avoiding temperature-crashes. Recordings of descending axons showed that conduction velocity was temperature dependent with a Q_{10}=1.39±.10 (n=4), and that action potential duration had a Q_{10}= 0.61±0.14 (n=4) for the range of 24-34^{o} C. This study shows that locomotor rhythm is highly temperature-dependent, but that pattern and other locomotor features have great stability in a large temperature range, suggesting they rely on a robustly hardwired networks. Axonal conduction velocity and action potential duration may contribute to locomotor effects, but the larger Q_{10} for locomotor frequency suggests participation of additional factors. Temperature effects are associated with diverse metabolic states, which may determine different operational modes of neural networks for adaptation to changes in ambient temperature.

Disclosures: J. Benedict: None. **A.E. Talpalar:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

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Title: The anatomical substrate of precise timing in zebra finch HVC

Authors: *J. KORNFELD[1], S. BENEZRA[2], R. T. NARAYANAN[3], F. SVARA[1], M. OBERLAENDER[3], W. DENK[1], M. A. LONG[2];
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Abstract: The sequential activation of neurons has been observed during a range of behaviors and cognitive states and is central to many models of neural circuit function, but the synaptic

connections enabling such dynamics are poorly understood. Song production in the zebra finch depends on a cortical region called HVC, which contains a major class of excitatory ($HVC_{(RA)}$) neurons that fire action potential bursts in a fixed, sequential pattern during singing. These neurons not only project to downstream motor centers but also make numerous connections within HVC and could, therefore, form a sequence-generating synaptic chain. Evidence for this network architecture has, however, been either indirect or inconclusive. Here we employ light and transsynaptic electron microscopy to explore the synaptic connectivity of $HVC_{(RA)}$ neurons. We find that $HVC_{(RA)}$ cells receive the vast majority of their excitatory connections from distal sites on the axons of other $HVC_{(RA)}$ cells; proximal axonal sites, on the other hand, nearly always target inhibitory interneurons. Overall, this connectivity pattern provides evidence for a distributed excitatory synaptic chain supported by local inhibition, characteristic of coupled winner-take-all architectures.

Disclosures: J. Kornfeld: None. **S. Benezra:** None. **R.T. Narayanan:** None. **F. Svara:** None. **M. Oberlaender:** None. **W. Denk:** None. **M.A. Long:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

Location: Halls B-H

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Topic: E.07. Rhythmic Motor Pattern Generation

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Kavli Institute for Brain and Mind

Title: Synapse distribution on leech neurons reconstructed via electron microscopy

Authors: *J. PIPKIN[1], M. H. ELLISMAN[2], W. B. KRISTAN, Jr.[2];
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Abstract: The anatomical distribution of synaptic inputs and outputs within a neuron's arbor constrains that neuron's behavior. In many invertebrate systems, neurons assemble into circuits by extending their arbors into regions of neuropil, where they form appropriate synapses despite dense intermingling with many possible synaptic partners. We describe the patterns and distribution of synaptic contacts among arbors of neurons from the segmental ganglia of the

medicinal leech, *Hirudo verbana.* To locate sites of synaptic input and output, we reconstructed individual neurons from within a large volume of images spanning an entire juvenile leech ganglion generated via serial blockface scanning electron microscopy. We find three basic patterns governing the distribution of synaptic inputs and outputs. In some neurons, inputs and outputs are evenly distributed throughout all branches of the arbor. In others, the ipsilateral half of the arbor contains only inputs while the contralateral half contains both inputs and outputs. Finally, some cells contained only synaptic inputs or very few outputs at least within the ganglion-contained neuropil. We also analyzed specific pairs of synaptically-linked neurons and found that their synapses were broadly distributed over the region of overlap between the output-containing portion of the presynaptic neuron's arbor and any portion of the postsynaptic neuron's arbor. These results have implications for both functional and developmental models of circuit function in neuropilar systems.

Disclosures: J. Pipkin: None. **M.H. Ellisman:** None. **W.B. Kristan:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 535.15/CCC5

Topic: E.07. Rhythmic Motor Pattern Generation

Title: Connectomic analysis of the local wiring in L6 of Mouse primary motor cortex

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Abstract: Long range projecting neurons in the deeper cortical layers 5 and 6 of primary motor cortex are one of the principal inputs to the cortico-striatal pathways. Here we used 3D EM microscopy and circuit reconstruction to analyze the local circuit in Layer 6 of mouse primary motor cortex. A volume of 93 × 74 × 108 μm^3 was imaged using serial block face scanning electron microscopy (SBEM) with a resolution of 12 x 12 x 28 nm^3 and analyzed using semi-automated segmentation (SegEM, Berning et al., 2015) . We are currently quantifying the degree of specificity of axonal wiring with respect to the different types of postsynaptic targets present, a first step to establish a baseline of healthy L6 connectomes. This may open the path to analyse specific wiring changes in mutant mouse models of psychiatric diseases.

Disclosures: J. Straehle: None. **M. Helmstaedter:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

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Program#/Poster#: 535.16/CCC6

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF-CRCNS Grant DMS-1430077

US-Israel Binational Science Foundation Grant 2011059

Title: Endogenous rhythm and pattern-generating circuit interactions in Periplaneta americana motor centers

Authors: *I. DAVID[1], P. HOLMES[2,3], A. AYALI[1,4];
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Abstract: Cockroaches are known for their rapid and stable locomotion. The walking and running motor pattern is a product of the intricate interplay between central-pattern-generator (CPG) networks and sensory feedback that shapes their rhythmic motor output. The cockroach is a leading model in studies of motor pattern generation and a useful inspiration for technological innovations. This study was aimed at characterizing the basic-endogenous rhythmic activity generated by isolated cockroach thoracic pattern-generating circuits, to fill current gaps in our knowledge, re-examine previous findings, and establish general rules for the system connectivity. We monitored pilocarpine-induced simultaneous rhythmic motor output of levator and depressor motorneurons (MNs) in the meso- and meta-thoracic segments of brainless and deafferented cockroach preparations. Data analyses included measures of coupling strength among the network units, phase relations, latencies between and overlaps of rhythmic bursts of activity, spike frequencies within bursts, and the dependence of these parameters on burst frequency. Our findings reveal an endogenous front-to-back activation sequence typical of double-tripod locomotion. Differences in the activity of homologue units in the different segmental ganglia were observed, as well as asymmetries in connectivity among the different components. The data suggest differences in the functional role of network units and unit interactions in the overall rhythm generation. Many of the observed characteristics were similar to those exhibited by intact animals, suggesting a dominant role for feedforward control in cockroach locomotion, while differences provide better understanding of the role of sensory feedback in locomotive behavior. Based on our data we posit an explanatory connectivity scheme within the hemisegmental levator-depressor CPG's components and among neighboring homologue CPGs. We combine our experimental data with results and assumptions drawn from earlier studies in order to present a more complete connectivity scheme, which includes the

subesophageal ganglion and the prothoracic hemiganglia. The proposed parsimonious model can explain our data without compromising the system's ability to generate different locomotion-related behaviors, and provides a useful tool for further discussion of our current findings, as well as guiding future work. Our data also enable more comprehensive comparisons with data obtained from other insects that, while sharing their basic thoracic neural architecture with the cockroach, produce very different locomotion patterns under similar experimental conditions.

Disclosures: I. David: None. **P. Holmes:** None. **A. Ayali:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 535.17/CCC7

Topic: E.07. Rhythmic Motor Pattern Generation

Support: UDG, Biomedical Sciences PhD program

Title: Glutamate, CNQX or WAY-10063 produce fictive locomotion in thalamic cats

Authors: *S. H. DUENAS JIMENEZ[1], L. LOPEZ VALENZUELA[2], I. JIMENEZ ESTRADA[4], M. E. MENDOZA GARRIDO[4], B. DE LA TORRE VALDOVINOS[3], L. CASTILLO HERNANDEZ[5], J. M. DUEÑAS JIMENEZ[2], N. E. FRANCO RODRIGUEZ[6]; [1]Univ. De Guadalajara, Zapopan, Mexico; [2]Physiol., [3]Computat. Sciences, CUCEI, Univ. de Guadalajara, Guadalajara, Mexico; [4]Physiology, Biophysics and Neurosci., CINVESTAV-IPN, Mexico City, Mexico; [5]Physiol. and Pharmacol., Univ. de Aguascalientes, Aguascalientes, Mexico; [6]Computat. Sci., Univ. of Guadalajara, Guadalajara, Mexico

Abstract: In thalamic and subsequently spinalized cats we analyzed whether glutamate (GLU), CNQX (an AMPA/Kainate glutamate inhibitor drug), serotonine (SER), or WAY-10063 a serotonin 1A receptor inhibitor drug injected: intra brain stem (BS, 1 mm down the obex) or in spinal cord (ISC) interfered with fictive locomotion (FL) or fictive scratching (FS) produced by pinna stimulation. In twelve cats, brain cortex was ablated under brevital anesthesia, we named these cats: thalamic cats (TC). Two hours later, the same animals were spinalized cutting the spinal cord at C1-C2 segment; spinalized cats (SC). In these cats once tubocurarine (0.25-01mM) was topically applied in C1-C2 segments, pinna stimulation induces fictive locomotion (n=3) or FS (n=6). Glutamate in BS or ISC induces the FS-FL phenomena. It also increase the electroneurogram (ENG) extensor burst duration during spontaneous fictive locomotion (n=2). In three TC, CNQX was injected (5nM/min) in BS or ISC at L3-L4 segments. CNQX in BS induced FL. In TC, SER injected in BS or ISC increase the scratching episodes duration and the

extensor ENG amplitude during FL. In other three different cats, which pinna mechanical stimulation did not elicited FS or the FS-FL phenomena, WAY-10063 (0.2-3.5 nM/min,) in the BS or injected i.v. (0.25 mg/kg (n=2) induces FL. In spinal cats, glutamate (n=3), CNQX (n=3), SER (n=3) and GLU (n=3) favored pinna stimulation to generate FS. Activation of BS neurons with NMDA receptors could form a pathway activating the spinal cord locomotion generator. These neurons localized between obex and C1 spinal cord segment are also activated by d-tubocurarine and pinna stimulation and could be inhibited by serotonin since WAY-10063 a SER 1A receptor inhibitor injected i.v. or in BS favored pinna stimulation to generate FL.

Disclosures: S.H. Duenas Jimenez: None. **L. Lopez Valenzuela:** None. **I. Jimenez Estrada:** None. **M.E. Mendoza Garrido:** None. **B. de la Torre Valdovinos:** None. **L. Castillo Hernandez:** None. **J.M. Dueñas Jimenez:** None. **N.E. Franco Rodriguez:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: Swedish research council

Karolinska Institute

Swedish Brain Foundation

Title: Functional diversity of excitatory V0 interneurons in the adult zebrafish

Authors: R. BJÖRNFORS, *A. EL MANIRA;
Karolinska Inst., Stockholm, Sweden

Abstract: Flexibility in the bilateral coordination of muscle contraction underpins variable locomotor movements or gates. The left-right coordination during locomotion can vary in a context-dependent manner to produce alternation through the activity of commissural interneurons connecting local interacting circuits on the two sides of the spinal cord. The V0 interneurons represent a major commissural neuronal population comprising dorsal excitatory (V0v) and ventral inhibitory (V0d) classes. The current view of V0 function derives from studies in immature motor systems (larval zebrafish and newborn mouse), but the activity patterns of V0d and V0v classes is unknown. Here we examined how the activity of the V0v interneurons varies with the speed of locomotion in adult zebrafish. Our results show that although V0v interneurons express a defined transcription factor and transmitter phenotype, their activity

patterns during locomotion are heterogeneous. V0v interneurons could be segregated into two distinct types based on whether or not they display rhythmic activity during locomotion. The rhythmically active V0v interneurons could be further subdivided into three main sub-classes engaged sequentially during swimming, first at slow then intermediate and finally fast locomotor speeds. The order of recruitment of the three sub-classes of V0v interneurons is defined by a combined computation of their synaptic drive and intrinsic properties. The extent of the rhythmic excitation is the result of a scaling of the synaptic current with the input resistance of the different V0v interneurons. This study thus uncovers, for the first time in an adult vertebrate, an important organizational principle for a key class of commissural interneurons and the underlying cellular and synaptic mechanisms defining their pattern of recruitment as a function of locomotor speed.

Disclosures: **R. Björnfors:** None. **A. El Manira:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

Location: Halls B-H

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Program#/Poster#: 535.19/CCC9

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Boehringer Ingelheim Fonds PhD Fellowship

Title: Wiring-specificity in a larval zebrafish premotor network

Authors: ***F. SVARA**[1], J. KORNFELD[2], W. DENK[2], J. BOLLMANN[3];
[2]electrons - photons - neurons, [1]Max Planck Inst. of Neurobio., Martinsried, Germany; [3]Dept. of Biomed. Optics, Max Planck Inst. for Med. Res., Heidelberg, Germany

Abstract: Larval zebrafish produce a range of different motor patterns, appropriate to the range of different situations they face. Noxious stimuli induce vigorous and fast tail bending, followed by a period of high-frequency tail beating, to allow a quick escape. On the other hand, the fish produces precise, low-amplitude, low-frequency swim patterns when it pursues its prey. Central pattern generator circuits (CPGs) in the spinal cord underlie the generation of these movements. A segmentally repeating pool of motoneurons (MNs) is recruited in an orderly fashion, with the smaller, more ventral MNs engaged during the weaker swims and large, dorsal MNs being added only during the strongest movements.
How the spinal CPG interneurons produce this graded activation of MNs is not well understood, but it is known that ipsilateral descending interneurons (CiDs) provide rhythmic excitation to MNs and commissural inhibitory interneurons ensure left-right alternation. These inputs interact

with the intrinsic excitabilities of the MNs, with larger MNs being less excitable.
In a stochastic connectivity model, the recruitment of MNs would be governed mainly by their input resistance. Alternatively, in a wiring-based recruitment model, subtypes of premotor interneurons would precisely select their postsynaptic partners from the pool of MNs.
To distinguish between these models, we reconstructed the MN pool in one segment of a 6 day old larval zebrafish from a Serial Block-Face Electron Microscopy dataset containing 2.5 segments of spinal cord, allowing us to comprehensively elucidate the distribution of MN subtypes. We then traced from synaptic terminals on selected MNs of different types back into their presynaptic partners, identifying the CPG elements that activate and inhibit them, including the CiD and CoBL interneurons.
We find that a large subset of the CiDs is highly specific to the type of MN it contacts and that the somata of CiD subtypes with different specificity are dorso-ventrally segregated.

Disclosures: **F. Svara:** None. **J. Kornfeld:** None. **W. Denk:** None. **J. Bollmann:** None.

Poster

535. Rhythmic Motor Patterns: Connectivity

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: Swedish Research Council

Karolinska Institute

Swedish Brain Foundation

Title: Convergence rules and synaptic fidelity dictate the recruitment of motor neurons in adult zebrafish

Authors: *J. SONG, A. EL MANIRA;
Karolinska Inst., Stockholm, Sweden

Abstract: Locomotor movements are generated by spinal locomotor networks and require the coordinated recruitment of motor neuron pools to drive muscle contractions in an appropriate sequence. Different motor units need to be engaged successively from slow to intermediate and fast to increase the locomotor speed. Presumably, specific rules of convergence of synaptic drive from premotor excitatory interneurons are needed to dictate the sequence of activation of motor neurons. However, the nature of the convergence and the dynamics of the excitatory inputs to identified motor neurons are still unclear. We have addressed these issues in the adult zebrafish

in which the locomotor circuit is organized in three sub-circuit modules with sub-classes of the excitatory V2a interneurons connecting selectively to slow, intermediate or fast motor neurons. We sought to determine the rules of convergence of V2a interneurons that reliably control the recruitment of motor neurons of the slow, intermediate and fast sub-circuits. Our results show that the convergence rules varied among the three sub-circuits. In addition, there were differences within each sub-circuit depending on the firing properties of the presynaptic V2a interneurons. Indeed, V2a interneurons either displayed intrinsic burst or tonic firing. The bursting V2a interneurons target the motor neuron dendrites to elicit large EPSPs, while the tonically firing ones have bifurcating axons targeting the soma to produce relatively smaller EPSPs. During each V2a interneuron burst there was super linear EPSP summation that was highest in the slow sub-circuit module and lowest in the fast module and required activation of NMDA receptors. These properties ensured the fidelity and strength of the transfer of excitatory drive within each sub-circuit module and hence a module-specific convergence rule. In the slow module the convergence of only two V2a interneurons was required to engage the corresponding motor neurons. The intermediate module required the convergence of 6 V2a interneurons, while the fast module necessitated more than 40 interneurons to ensure motor neurons recruitment. Thus, this study reveals the rules of convergence that in combination with the NMDA-induced super linear synaptic dynamics ensure the sequential activation of motor neurons of three sub-circuit modules to increase the locomotor speed.

Disclosures: J. Song: None. **A. El Manira:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: ANR-RythmDev

AP-HM

Title: Brainstem functions assessment in a cohort of very preterm babies born at less than 28 weeks

Authors: *J.-C. VIEMARI, A. SAMONINI, B. BELLOT;
CNRS-INSTITUT DE NEUROSCIENCES DE LA TIMONE, Marseille, France

Abstract: Although significant advances in neonatal care have increased survival rates of preterm infants born before 28 weeks gestation, a concomitant decrease in neuro developmental disorders has not been achieved. Cerebral injuries, well documented during the previous years, in preterm babies are particularly deleterious since they occur in a developing brain. They affect both white and grey matter by complex mechanisms and the principal targets are the developing oligodendrocytes and neurons of the subplate. All these criteria define the encephalopathy of prematurity. Nevertheless, the consequences of prematurity at the level of the brainstem are not very well known and may explain neuro-developmental disorders with normal RMI.
The assessment of the motor repertoire is complementary to the neurological examination and may represent a diagnostic tool for cerebral palsy, mild motor deficits and delayed acquisition in children. The newborn have a rich motor repertoire. GMs play a key role in the development due to the feedback that they send to cortical neurons and reflect the maturational stage of the CNS. Lesions of the brainstem caused by prematurity may induce alterations of the motor repertoire. Dysautonomic disorders, such as bradycardia, apneas, feeding problems that occur frequently in very preterm babies may reflect brainstem abnormalities. These symptoms are also described in other pathologies such as in Rett syndrome, in ASD and SIDS. In these pathologies, deficits of the 5-HT system have been described and associated with dysautonomia. It would then be interesting to evaluate 5-HT levels in very preterm babies.
The serotonergic system develops very early during gestation and is one of the first neurotransmitter to appear in the developing brain. The main 5-HT nuclei are located within the brainstem. 5-HT plays an important role in the homeostasis and the modulation of the respiratory network. Moreover, previous studies have shown that 5-HT projections to the spinal cord are involved in posture and in the coordination. It is tempting to think that 5-HT deficits may have some repercussions on the development of the CNS, changing activity dependent processes, such as spontaneous activity recorded at the spinal level in rodents.
Here we compared 5-HT platelets level in preterm infants born before 28 weeks with newborn infants. We would like to correlate 5-HT levels with MRI of the posterior fossa, the GMs and different parameters of dysautonomia such as cardiac variability, succion-swallowing and respiratory patterns.

Disclosures: **J. Viemari:** None. **A. Samonini:** None. **B. Bellot:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 536.02/CCC12

Topic: E.07. Rhythmic Motor Pattern Generation

Title: Postnatal development of spinal circuits in the presence and absence of descending systems

Authors: *C. C. SMITH[1], J. F. R. PATON[2], S. CHAKRABARTY[1], R. M. ICHIYAMA[1]; [1]Univ. of Leeds, Leeds, United Kingdom; [2]Univ. of Bristol, Bristol, United Kingdom

Abstract: Interestingly, neonatally spinalised (TX) rats display enhanced prospects of locomotor recovery compared to adults. Both these models must learn to produce hindlimb stepping with only sensory input, suggesting a fundamental difference in the ability of the neonatally TX spinal cord to utilise this information. This difference is likely induced by distinct developmental activity patterns which alter the organisation of crucial spinal circuits. However, the postnatal (PN) development of the sensory-motor circuitry isolated from descending influences is not well understood. This study assessed the development of lumbar spinal circuits and sensory systems of normal and neonatally (PN5) TX rats. All assessments were performed at PN10, 14 and 21. For the transection group, PN5 rats were anaesthetised (Isoflurane) and spinally transected (mid-thoracic), after which they were allowed to recover for 5 (PN10), 9 (PN14) or 16 (PN21) days. Immunohistochemistry was used to assess PN development of proprioceptive afferents (PA) and their presynaptic innervation by GABApre neurons (P boutons). PA were quantified with respect to inputs to motoneurones (MNs), Renshaw cells and their laminae distribution. Electrophysiological (H-reflex) assessments were used to physiologically corroborate the anatomical findings. Finally, excitatory and inhibitory premotor inputs to MNs were quantified to assess the impact of PN5 TX on development of the intraspinal circuitry. Results show that during normal development, proprioceptive afferents are retracted from the lumbar spinal cord, whilst GABApre (P bouton) innervation of PA afferents increased with development. Premotor projections were retracted. PN5 transection abolished afferent retraction and severely attenuated P bouton proliferation on PA terminals. H-reflex data showing increased excitability and reduced modulation of the monosynaptic reflex affirmed the anatomical data. Importantly, normal development of premotor projections was utterly resilient to PN5 transection. This study shows that PN retraction of PA is abolished following neonatal TX, leading to both disruption and maintenance of the formation of key spinal circuits in the absence of descending systems. These changes may represent crucial differences between neonatal and adult spinal cord injuries which afford enhanced recovery to the former. Understanding how spinal circuits compensate for developmental loss of supraspinal control is vital to directing questions regarding future and current treatments for conditions such as cerebral palsy and spinal cord injury.

Disclosures: C.C. Smith: None. **J.F.R. Paton:** None. **S. Chakrabarty:** None. **R.M. Ichiyama:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

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Program#/Poster#: 536.03/CCC13

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF 1454904

Title: The contribution of central and sensory information in locomotor recovery after injury to the CNS

Authors: *J. G. PUHL[1], M. M. NEWHOFF[2], M. C. P. RUE[3], A. W. BIGELOW[3], K. A. MESCE[4];
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Abstract: We have shown previously that the medicinal leech (Hirudo verbana) exhibits a remarkable ability to recover its crawling after coordinating signals from the brain are removed. Such descending information is vital for the initiation and coordination of body segments during crawling in uninjured control animals. The aim now is to determine what changes occur after injury that facilitates the recovery of coordinated crawling in the absence of descending information. To investigate how crawling becomes reinstated, we examined the neuroanatomical, electrophysiological and cell-molecular features of the anterior-most ganglion below the transection, as well as the rest of the nerve cord in recovered animals. We determined that recovery does not involve the reconnection of axons from anterior neuronal projections. Furthermore, major morphological alterations of motoneurons were deemed not to contribute substantially to recovery. One hypothesis we tested was whether the dopamine (DA)-sensitive crawl neural networks in the lead ganglion of recovered leeches were newly capable of providing both crawl drive and coordination to neighboring crawl oscillators. Although DA administered only to the lead ganglion was found to be effective in generating crawl-bursting within neighboring segmental oscillators, the phase relationships were aberrant and lacked the metachrony specific to crawling. In recovered nerve cords in which all the ganglia were bathed in DA, a spectrum of crawl-bursting profiles emerged. Even though some of the nerve cords from crawl-recovered animals showed a few episodes of crawl-specific phase relationships, none of the 12 preparations tested displayed continuous bouts of crawl-specific coordination similar to that seen in isolated nerve cords with their brains connected. Thus, fictive crawling with appropriate segmental phase relationships appears to depend on the presence of the body and its ability to provide sensory input, in contrast to controls. Thus, our next steps are focusing on the role of proprioceptive feedback arising from body-wall stretch receptors. Neurobiotin fills of stretch receptors in recovered and control leeches reveal some differences in the dye-coupling of

these cells. Semi-intact preparations will be used next to test whether crawl-specific coordination can be established when the stretch receptors are activated during uncoordinated crawling in recovered animals. Lastly, we have begun to investigate changes in gene transcript levels in response to injury by collecting, sequencing and quantifying levels of RNA from entire ganglia of recovered and control leeches.

Disclosures: J.G. Puhl: None. **M.M. NewHoff:** None. **M.C.P. Rue:** None. **A.W. Bigelow:** None. **K.A. Mesce:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 536.04/CCC14

Topic: E.07. Rhythmic Motor Pattern Generation

Support: CIHR

Title: Functional contribution of glutamatergic neurons of the medullary reticular formation to the motor control in mice

Authors: *M. LEMIEUX, F. BRETZNER;
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Abstract: The motor activity of limbs is generated within the spinal cord network but is initiated and modulated by supraspinal centers. Due to its intermediate position between the mesencephalic locomotor region (MLR) and the spinal interneuronal circuit, the medullary reticular formation (MRF) is bound to play a crucial role in the locomotor control. However, the identity of MRF neurons involved in motor control is still unclear. Using optogenetic tools accessible in the mouse, our goal was to study the functional contribution of MRF glutamatergic neurons to motor and locomotor control. Optical probes were inserted chronically into one side of the MRF of VGluT2-cre mice expressing Channelrhodopsin-2, a photo-activated cationic channel. The EMG activity was recorded from both flexors (Tibialis anterior, TA) and extensors (Gastrocnemius lateralis, GL) of the ankle at rest and during locomotion upon a 10 or 80 ms photo-stimulation . At rest, a 10 ms photostimulation evoked excitatory responses in TA and GL on both sides. As the intensity of photostimulation increased, the failure rate and the latency decreased, whereas the duration and amplitude of the EMG responses increased. Responses were evoked at the lowest threshold and the shortest latency in the ipsilateral TA. During locomotion, a 10 ms photostimulation evoked excitatory responses in TA and inhibitory ones in GL during the stance phase, while it evoked excitatory responses in the GL and either excitatory or no

responses in the ipsilateral TA during the swing phase. Inhibitory responses were sometime observed in the contralateral TA. An 80 ms photostimulations increased the duration of the ongoing step cycle, but without influencing the subsequent step cycles. We conclude that glutamatergic neurons from the MRF act on flexor and extensor muscles at rest, influence the locomotor pattern but not the rhythm.

Disclosures: M. Lemieux: None. **F. Bretzner:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 536.05/CCC15

Topic: E.07. Rhythmic Motor Pattern Generation

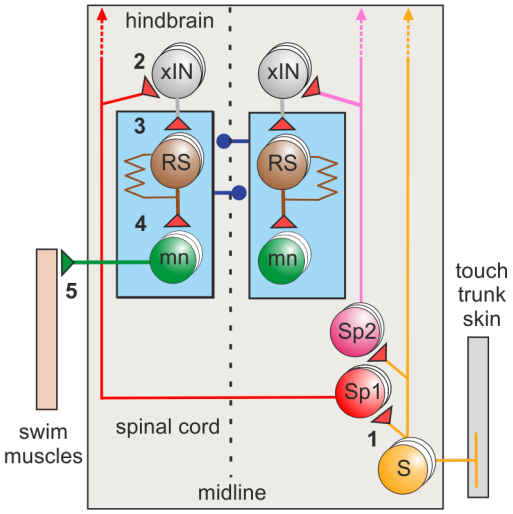
Support: BBSRC

Title: Variable delays in the decision to move: neurons coordinating the sensory initiation of locomotion

Authors: *S. KOUTSIKOU[1], E. BUHL[1], R. MERRISON-HORT[2], R. BORISYUK[2], S. SOFFE[1], A. ROBERTS[1];
[1]Univ. of Bristol, Bristol, United Kingdom; [2]Univ. of Plymouth, Plymouth, United Kingdom

Abstract: Sensory stimuli can lead to coordinated movements like eye saccades or locomotion where reaction times are long (>100ms) and variable. The current hypothesis is that decisions to act involve the noisy build-up of excitation to a threshold as sensory information is 'integrated' (Gold & Shadlen 2007 *Ann Rev Neurosci* 30:535). Where and how this happens remains unknown. Mammal nervous systems are very complex but all animals must decide whether to respond. We study hatchling *Xenopus laevis* tadpoles where most neurons in the pathway from skin touch to swimming are known. Delays to swimming are long and variable in behaving tadpoles (45-250ms) and in motor nerve recordings from immobilised tadpoles (20-140ms). Whole-cell recordings show synaptic input and spiking in neurons of the swim initiation pathway and the reticulospinal neurons (RS) that drive swimming (Li et al. 2010 *JNeurosci* 30:16609). After a brief skin stimulus, EPSPs build up slowly and noisily in RSs on both sides. The first side to reach threshold fires after 20-60ms and swimming follows. The known sensory (S) and sensory pathway (Sp) neurons fire once at 5-15ms so cannot explain the slow EPSP build-up. We record from hindbrain 'extension' neurons (xINs) which are excited at short latencies (5-16ms) by skin stimuli. They start spiking at 8-30ms but unlike Sps fire repetitively to extend excitation and introduce variability. A network model of 30 xINs with mutual excitation can also extend firing

to brief stimulus. The pathway from sensory stimulus to swimming therefore has 5 functional layers: (1) sensory neurons (S); (2) sensory pathway neurons (Sp); (3) extension neurons (xINs) prolong firing and introduce variability, (4) reticulospinal neurons (RS) make the decision to swim when xIN excitation sums to threshold and recruits the whole electrically-coupled RS population; RSs excite (5) motoneurons (mn) which excite the muscles. We conclude that the long and variable delays to swimming, as widely observed in decisions to act, result from the noisy slow-rising excitation in RSs which is a consequence of the newly discovered xIN layer.



Disclosures: **S. Koutsikou:** None. **E. Buhl:** None. **R. Merrison-Hort:** None. **R. Borisyuk:** None. **S. Soffe:** None. **A. Roberts:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 536.06/CCC16

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSERC

Title: Short-interval intracortical inhibition projecting to the biceps brachii during arm cycling

Authors: ***L. R. ALCOCK**[1], E. J. LOCKYER[1], A.-J. SPENCE[1], K. E. POWER[2,3];
[2]Human Kinetics and Recreation, [1]Mem. Univ. of Newfoundland, St Johns, NL, Canada; [3]Fac. of Med., St. John's, NL, Canada

Abstract: The motor cortex has been previously shown to play a role during spinally-mediated rhythmic and alternating locomotor outputs. Recent studies likewise suggest that evidence of enhanced corticospinal excitability in the absence of increased spinal excitability during arm cycling indicates that excitatory output is mediated via changes at the supraspinal level. Short-interval intracortical inhibition (SICI) is a type of cortical inhibition which is elicited through a paired-pulse transcranial magnetic stimulation (TMS) paradigm and is considered to be represented by a reduction in test motor-evoked potential (MEP) amplitude. The present study sought to explore the possible involvement of SICI projecting to arm muscles during arm cycling. Ten volunteers (eight male, two female) participated in this study. Subjects completed the protocol using a SCIFIT arm cycle ergometer during which muscle responses were recorded via surface electrodes placed on the biceps brachii. SICI was assessed using a subthreshold conditioning stimulus (CS) and a suprathreshold test stimulus (TS) made relative to active motor threshold (AMT) at an interstimulus interval (ISI) of 1.5ms applied over the dominant motor cortex. Responses were elicited using conditioning stimuli of 70% and 90% of AMT during arm cycling at a constant workload of 25W and 60RPM. We have previously reported enhanced MEP responses due to supraspinal factors during the flexion phase of cycling, therefore subjects received stimulation at this phase throughout the study. SICI was effectively elicited in all subjects; test MEP amplitudes were significantly reduced by 72.5% ($p = 0.003$) and 66.9% ($p = 0.004$) following conditioning stimuli of 70% and 90% AMT, respectively. Background EMG recordings show no significant variance between test and conditioned trials, indicating similar intensity of muscle activation was used throughout the experiment. This data represents the novel finding that SICI is present during arm cycling, which suggests that activation of inhibitory intracortical interneurons may modify the descending supraspinal drive to muscles during arm cycling.

Disclosures: **L.R. Alcock:** None. **E.J. Lockyer:** None. **A. Spence:** None. **K.E. Power:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

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Program#/Poster#: 536.07/CCC17

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSERC

Title: Workload-dependent changes in corticospinal excitability to the elbow extensors during arm cycling

Authors: *E. J. LOCKYER[1], D. A. FORMAN[1], D. T. G. PHILPOTT[1], D. C. BUTTON[1,2], K. E. POWER[1,2];
[1]Sch. of Human Kinetics and Recreation, Mem. Univ. of Newfoundland, St John's, NL, Canada; [2]Fac. of Med., Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: The purpose of the present study was to investigate the influence of different external workloads on the modulation of supraspinal and spinal excitability to the triceps brachii during arm cycling. Ten male participants (age, 24.3 ± 5.3 years; weight, 81.7 ± 7.3 kg) participated in this study. Supraspinal and spinal excitability were assessed using transcranial magnetic stimulation (TMS) of the motor cortex and transmastoid electrical stimulation (TMES) of the corticospinal tract, respectively. Motor evoked potentials (MEPs) elicited by TMS and cervicomedullary-evoked potentials (CMEPs) elicited by TMES were measured from the triceps brachii at two different positions corresponding to mid-elbow flexion and mid-elbow extension (6 and 12 o'clock made relative to a clock face, respectively) while arm cycling at 10, 20, 30, and 40 W and a set cadence of 60 rpm. Electromyographic (EMG) recordings were made from the dominant arm triceps brachii. MEP amplitudes (n = 10) were not statistically different as cycling workload increased during either flexion (P = .266) or extension (P = .366). Similarly, CMEP amplitudes (n = 5) were not statistically different as cycling workload increased during either flexion (P = .489) or extension (P = .783). In addition, MEP amplitudes were not different between flexion and extension (P = .435). However, CMEP amplitudes were larger (P = .016) during flexion (10W, 18.7% ± 5.05%; 20W, 18.9% ± 5.97%; 30W, 24.1% ± 5.96%; 40W, 21.58% ± 4.69% of triceps brachii M_{max}) than during extension (10W, 10.0% ± 4.45%; 20W, 7.67% ± 3.34%; 30W, 9.7% ± 4.03%; 40W, 10.3% ± 3.65% of triceps brachii M_{max}). The data indicate that corticospinal excitability projecting to the triceps brachii did not increase during arm cycling with increased workload at either position. However, spinal excitability as workload increased demonstrated phase-dependent modulation (i.e., increased during elbow flexion and decreased during elbow extension).

Disclosures: E.J. Lockyer: None. **D.A. Forman:** None. **D.T.G. Philpott:** None. **D.C. Button:** None. **K.E. Power:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSERC

Title: Corticospinal excitability to the biceps brachii is elevated during arm cycling with a neutral versus pronated forearm position

Authors: *D. FORMAN[1], G. N. FORMAN[2], M. RICHARDS[3], M. W. R. HOLMES[2], K. E. POWER[3,4];
[1]Fac. of Sci., Univ. of Ontario Inst. of Technol., Whitby, ON, Canada; [2]Fac. of Hlth. Sci., Univ. of Ontario Inst. of Technol., Oshawa, ON, Canada; [3]Sch. of Human Kinetics and Recreation, [4]Fac. of Med., Mem. Univ., St. John's, NL, Canada

Abstract: The influence of forearm position on corticospinal excitability (CSE) to the biceps brachii has previously been assessed during static, resting conditions. However, the relationship between forearm position and CSE has not been examined during a rhythmic motor output. Therefore, the purpose of this study was to examine the influence of neutral and pronated forearm positions on CSE to the biceps brachii during arm cycling. Corticospinal and spinal excitability were measured using motor evoked potentials (MEPs) elicited via transcranial magnetic stimulation (TMS) and cervicomedullary-evoked potentials (CMEPs) elicited via transmastoid electrical stimulation (TMES), respectively. Recordings were taken from the biceps brachii during mid-elbow flexion and extension (6 and 12 o'clock positions relative to a clock face) while arm cycling with two different forearm positions; neutral and pronated. Arm cycling was kept at a constant cadence of 60 rpm and a fixed workload of 5% of the individual's cycling peak power. Recordings were also taken during tonic elbow flexion of similar muscle activity in order to differentiate any findings as either dependent on forearm position or task. Peak-to-peak amplitudes were significantly larger at the 6 o'clock position while arm cycling with a neutral forearm position compared to pronated for both MEPs (Neutral: 55.6 ± 20.2% of M_{max}, Pronated: 38.2 ± 21.7% of M_{max}, $P < .01$) and CMEPs (Neutral: 31.3 ± 11.9% of M_{max}, Pronated: 24.1 ± 11.4% of M_{max}, $P < .005$). There were no differences in amplitude at the 12 o'clock position between forearm positions for MEPs ($P = .09$) or CMEPs ($P = .54$). For the tonic contractions, MEPs were significantly larger with a neutral versus pronated forearm position (Neutral: 40.1 ± 14.8% of M_{max}, Pronated: 30.4 ± 11.2% of M_{max}, $P < .05$) while there were no difference in CMEPs ($P = .38$). CSE was higher with a neutral forearm position for both arm cycling and tonic elbow flexion. While spinal excitability was also higher with a neutral forearm position during arm cycling, no difference was found during tonic elbow flexion. This suggests that the influence of forearm position on spinal excitability is not universal across motor outputs and may be specific to arm cycling (ie. task dependent).

Disclosures: D. Forman: None. **G.N. Forman:** None. **M. Richards:** None. **M.W.R. Holmes:** None. **K.E. Power:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 536.09/CCC19

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSERC

Title: The role of central drive and afferent feedback on corticospinal excitability during arm cycling

Authors: ***N. SORAN**[1], L. R. ALCOCK[1], E. J. LOCKYER[1], A.-J. SPENCE[1], C. P. CHAYTOR[1], D. C. BUTTON[1,2], K. E. POWER[1,3];
[1]Human kinetics and Recreation, Mem. Univ. of Newfoundland, St Johns, NL, Canada; [2]Fac. of Med., Mem. Univ. of Newfoundland, Memorial University of Newfoundland, NL, Canada; [3]Fac. of Med., Mem. Univ. of Newfoundland, St John's, NL, Canada

Abstract: We recently demonstrated that supraspinal excitability to the biceps brachii, an elbow flexor, was enhanced during the extension phase of arm cycling as cadence increased from 60 to 90 rpm. The purpose of the present study was to determine if the increase in surpaspinal excitability was due to the modulation of supraspinal drive and/or afferent feedback. Corticospinal excitability was measured using motor evoked potentials (MEPs) elicited via transcranial magnetic stimulation (TMS). Recordings were made from the biceps brachii of the dominant arm during mid-elbow extension (12 o'clock relative to a clock face) while arm cycling using three experimental tasks and two cadences (i.e. 60 and 90 rpm). The three tasks were: 1) bilateral arm cycling (BL; supraspinal drive and afferent feedback present); 2) unilateral, non-dominant arm cycling with the dominant arm moving passively (P; no supraspinal drive with afferent feedback present); and 3) unilateral, non-dominant arm cycling with the dominant arm at rest (R; no supraspinal drive or afferent feedback present). MEPs were normalised to M_{max} and were expressed as a percentage. Preliminary data analysis demonstrates significant main effects for cadence ($p = .017$) and tasks ($p = .007$). As expected, the peak-to-peak amplitudes were significantly larger during cycling at 90 vs 60 rpm (60 rpm: $9.7 \pm 3.2\%$ of M_{max}; 90 rpm: $16.1 \pm 4.6\%$ of M_{max}, $p=.017$). During non-dominant arm cycling at 90 rpm, corticospinal excitability to the biceps brachii of the dominant arm was highest when at rest (R: $26.45 \pm 23.3\%$ of M_{max}) followed by passive arm cycling (P: 15.0 ± 11.4 % of M_{max}) and finally during bilateral arm cycling (BL: $7.0 \pm 7.3\%$ of M_{max}). Changes in interhemispheric inhibition (IHI) may partially explain the current findings. Enhanced corticospinal excitability in the higher cadence may result from cross facilitation and/or reduced IHI between motor cortices as the limbs attempt to cycle synchronously. Reduced IHI may also contribute to the increase in

corticospinal excitability with the higher cadence in dominant arm as it becomes less active as the need to inhibit an inactive hemisphere may not be required.

Disclosures: **N. Soran:** None. **L.R. Alcock:** None. **E.J. Lockyer:** None. **A. Spence:** None. **C.P. Chaytor:** None. **D.C. Button:** None. **K.E. Power:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

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Program#/Poster#: 536.10/CCC20

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NINDS NS090595

NSF PolS 2014906

Title: Behaviors formed by the coordination of ingestive motor actions with breathing

Authors: ***S.-M. LIAO**, D. KLEINFELD;
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Abstract: Ingestive behaviors, which include chewing, licking and swallowing, require a precise temporal coordination among the participating muscles. They further require coordination with the respiratory rhythm to avoid interference with the upper airway. Here we electrophysiologically record from selected muscles involved in ingestive behaviors of the rat and study how the rhythmic pattern of orofacial movements relates to the inspiratory rhythm, generated by the preBötzinger complex (Smith 1991 Science), and to the chewing rhythms, generated in a more dorsal-medially located nucleus nearby reticular formation (Nozaki et al 1986 J Neurphysiol; Chandler & Tal 1985 J Neurosci). Licking occurs at 6 to 7 Hz (Travers et al. 1997 Neurosci Biobehav Rev). The concurrent respiration is primarily composed of basal breathing, at frequencies < 3 Hz, with relatively few sniffing cycles, which occur at frequencies > 5 Hz. We observe that protrusion of the tongue movement never phase-locks with basal breathing although it will phase lock with sniffing. This implies that the coupling between the preBötzinger complex and the premotor circuitry that controls rhythmic tongue movements is strongly nonlinear. In contrast to breathing, rhythmic motion of the masseter muscle that drives chewing is strongly phase-locked with motion of the tongue at all frequencies. This later finding is consistent with common premotor projections to both the hypoglossal motor nucleus and the trigeminal motor nucleus (Stanek et al. 2014 eLife).

Disclosures: **S. Liao:** None. **D. Kleinfeld:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NINDS F31NS089316-02

CIHR MT-5877

NINDS NS090595

NINDS NS058668

NSF PolS 2014906

Title: Brainstem control of exploratory nose motion in rodents

Authors: *A. KURNIKOVA[1], M. DESCHÊNES[3], D. KLEINFELD[2];
[1]Neurosciences, [2]Physics, UC San Diego, La Jolla, CA; [3]Univ. Laval, Québec, QC, Canada

Abstract: Rodents shift their nose from side to side while they actively explore their surroundings. This motor action is used to sample and presumably lateralize odors in order to guide navigation. Here we find that nose motion occurs both spontaneously during sniffing and in response to presentation of an odorant. Lateral motion of the nose is phase-locked to rhythmic breathing (Deschênes et al. 2014 Anatomical Record), such that the peak displacement of the nose occurs during inspiration. A second component of this motion is a slowly varying bias, which changes on a time-scale of 0.5 seconds. The bias is strongly driven by odorant presentation and furthermore, the nose will not deflect to a side if the corresponding nostril is blocked. We study premotor control of nose motion to further understand the generation of orofacial exploratory behaviors in synergy with the respiratory drive. Premotor nuclei provide input to motor neurons that are located in the dorsal lateral division of the facial motor nucleus (Deschênes et al. 2016 Neuron) that in turn contact the deflector nasi muscle (Deschênes et al. 2014; Haidarliu et al. 2015 Anatomical Record) to drive deflection of the nose. As a means to localize putative premotor nuclei, we use replication competent rabies virus to transsynaptically label secondary inputs to the deflector nasi muscle. In preliminary data, we identified multiple clusters of secondary labeled cells, including a group in the reticular formation caudal to the facial motor nucleus, i.e., the retrofacial area. Activation of cells in this group with glutamate microstimulation deflects the nose and activates the deflector nasi muscle. Further, a complete transsection of this area completely abolishes nose motion to the affected side. Cells in the retrofacial area send primarily glutamatergic projections to the facial motor nucleus (Deshenes et. al. in press). We thus use AAV-FLEX-ReaChR, which drives expression of a red shifted

channelrodopsin, to target the glutamatergic cells in the retrofacial area and determine the effect of their activation on nose motion. These ongoing studies will delineate the contribution of neurons cells in the retrofacial region to lateral motion of the nose and in relation to the respiratory drive and other orofacial motor actions.

Disclosures: **A. Kurnikova:** None. **M. Deschênes:** None. **D. Kleinfeld:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 536.12/CCC22

Topic: E.07. Rhythmic Motor Pattern Generation

Title: Effects of different motor patterns on the stability of isochronous serial interval production

Authors: ***W. QI**[1], A. MIURA[2], K. KANOSUE[3];
[1]Sports Sci., Waseda Univ., Tokorozawa-Shi, Japan; [2]Sports Sci., [3]Waseda Univ., Saitama, Japan

Abstract: Isochronous serial interval production (ISIP) inevitably accompanies with variability, which plays a crucial role in sports and music performances. However, the question where and how variability occurs still remains unknown. The purpose of this study is to elucidate variability of different ISIP motor patterns (tapping and pressing) and delineate the dominant factor of variability categorized in Collier & Ogden's model (2004). Eleven males and nine females in healthy condition participated in this experiment (20-30 years old, right-handed). Subjects sit at a table upon which one force plate was placed and did tasks of tapping or pressing 21 times (20 intervals) at one of nine time intervals (500ms, 750ms, 1000ms, 1250ms, 1500ms, 1750ms, 2000ms, 2500ms and 3000ms). The time and force of taps and presses were recorded with the force plate. Before each task, subjects were required to listen to the target inter-tap (press) intervals supplied with a metronome until they memorized it. Each task was tested twice and the order of tasks was randomized across subjects. Average inter-tap (press) intervals, their standard deviations and their coefficient of variations were calculated. Coefficient of variation increased with the increase in length of inter-tap (press) interval but the trends of increasing were significantly different between tapping and pressing tasks (Interaction: $P<0.05$). The coefficient of variation in tapping tasks at inter-tap intervals above 2000ms was larger than that at 500ms, while there was no difference among intervals for pressing tasks ($P<0.05$). Therefore, different motor patterns would influence the stability of ISIP only at relatively fast pace. Furthermore, standard deviations of timekeeping process, motor delay and drift were estimated according to Collier & Ogden's model. Standard deviation of timekeeping process shows significant difference at 500ms, 750ms, 1000ms and 1500ms inter-tap (press) intervals between tapping

tasks and pressing tasks ($P<0.05$), while no significance was found in standard deviation of motor delay and drift between two kinds of tasks ($P>0.05$). From these facts, we suggest that motor patterns influence the stability of short interval ISIP movement. Furthermore, the variability in short interval ISIP movement occurred in timekeeping process rather than those of motor delay and drift.

Disclosures: W. Qi: None. **A. Miura:** None. **K. Kanosue:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

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Program#/Poster#: 536.13/CCC23

Topic: E.07. Rhythmic Motor Pattern Generation

Support: R01NS0985387

Title: Organization of medullary reticulospinal inputs to thoracic descending commissural interneurons

Authors: *B. K. LAPALLO, M.-C. PERREAULT;
Emory Univ., Atlanta, GA

Abstract: We have previously described new organizational principles of the medullary reticular formation (MRF) whereby reticulospinal (RS) neurons in medial and lateral MRF preferentially activate hindlimb and axial motoneurons, respectively (Szokol et al. 2008, Szokol and Perreault 2009). We have also shown that, in the L2 segment, the differential control by mMRF and lMRF is mediated in part via specific connections to descending commissural interneurons (dCINs; Szokol et al. 2011). The goal of the present work is to investigate the rules that may underlie the specificity of the RS-dCINs connections. One possibility is that the recruitment of dCINs by mMRF and lMRF is segment-specific. If so this could be coded by differences in relative proportions of dCINs recruited. Here we are assessing the recruitment patterns of dCINs in the T7 segment because of the difference in motoneuron composition (axial only) compared to the L2 segment (axial and hindlimb). We combined electrical stimulation of the mMRF and lMRF and single-cell calcium imaging in the isolated brainstem-spinal cord neonatal (P0-P6) mouse preparation. The response patterns to mMRF and lMRF stimulation were assessed for 78 dCINs (n= 7). We found that 7% responded to mMRF stimulation only, 14 % responded to lMRF only, 75% responded to both mMRF and lMRF and 4% did not respond to either. Of the group that responded to both MRF regions, the majority (63%) were preferentially activated by lMRF stimulation (lMRF>mMRF). These proportions are comparable to the proportions reported

earlier for L2 dCINs (8%, 26%, 36% and 30%, respectively) particularly if we consider that our limit for response detection was lowered slightly compared to past studies. Altogether the present findings do not appear to support a segment-specific recruitment of dCINs by RS inputs. However, our experiments have not so far distinguished between dCINs of different transmitter phenotypes. Current experiments are addressing the issue by determining the relative proportions of $vGluT2^+$ vs $vGluT2^-$ (excitatory vs inhibitory) dCINs that respond to mMRF and lMRF stimulation in both T7 and L2 segments.

Disclosures: **B.K. Lapallo:** None. **M. Perreault:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: CINVESTAV funding (JQ)

CINVESTAV visiting professorship award (HH)

Title: In search for the interneurons implicated in the resetting of the locomotor rhythm by extensor group I afferents

Authors: L. E. DOMÍNGUEZ-RODRÍGUEZ[1], K. STECINA[2], D. L. GARCÍA-RAMÍREZ[1], E. MENA-AVILA[1], J. J. MILLA-CRUZ[1], L. MARTÍNEZ-SILVA[1], H. HULTBORN[3], *J. N. QUEVEDO[1];
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Abstract: Sensory information arising from limb movements is able to control the spinal locomotor circuitry for locomotion to adapt the motor pattern to demands of the environment. Stimulation of extensor group I afferents during the flexion phase of fictive locomotion produces a suppression of the ongoing flexion and initiation of the subsequent extension (resetting to extension; Conway et al Exp Brain Res 1987, 68: 643-56; Hultborn et al Ann N Y Acad Sci 1998; 860:70-82). During fictive locomotion, instead of the classical Ib non-reciprocal inhibition, stimulation of extensor group I afferents produced an oligosynaptic excitation in extensor motoneurons with latencies (~3 ms) compatible with 3 interneurons interposed (see Gossard et al, Exp Brain Res 1994, 98:213-28). We assume that some interneurons in this pathway actually belong to the rhythm-generating layer of the Central Pattern Generator (CPG), since it causes a

resetting of the rhythm.
The present work was aimed to identify the interneurons causing the resetting to extension and mediating the polysynaptic group I excitation during L-DOPA-induced fictive locomotion in the cat (and was a continuation of previous work; Stecina et al 2007, SfN poster 78.11).
Peripheral nerve dissection, laminectomy and decerebration were made under isofluorane anesthesia. Subsequently spinalization at C1 was performed. Fictive locomotion was induced by the injection of Nialamide (50 mg/kg) followed by L-DOPA (100 mg/kg). Ankle extensor motoneurons were recorded with K^+-acetate-filled micropipettes. Orthodromic extracellular field potentials (EFPs) and interneuron spikes were recorded in the intermediate nucleus and the ventral horn with Na^+-glutamate-filled micropipettes (to be able to cause tonic firing of interneurons).
We recorded candidate interneurons to mediate the resetting to extension based on the following criteria: i) rhythmic activation during the extension phase, ii) mono-, di-, or trisynaptic activation by extensor group I afferents, iv) polysynaptic activation by stimulation of contralateral flexor reflex afferents. Some interneurons were antidromically activated from an extensor motor nucleus and their spikes associated to negative EFPs in the same motor nucleus, suggesting they were last-order excitatory interneurons.
We conclude that interneurons recorded fulfill the characteristics to belong to the neuronal pathway activated by extensor group I afferents during locomotion. As the activity of interneurons exhibit the same pattern as the 'resetting to extension', they may belong to the rhythm generating layer of the CPG for locomotion.

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Poster

536. Rhythmic Motor Patterns: Control Mechanisms

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 536.15/CCC25

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH grant R01 NS081713

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Title: Computational modeling of effects of dorsal root stimulation on locomotor CPG

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Abstract: We studied the effects of sensory nerve stimulation on fictive locomotor activity to understand afferent control of the central pattern generator (CPG) in the isolated neonatal mouse spinal cord. In our experiments, fictive locomotor activity was evoked by bath application of N-Methyl-D-aspartate (NMDA), dopamine (DA) and serotonin. The lumbar dorsal roots (dL5) were stimulated while monitoring activity of flexor-related ventral roots, ipsi- (iL2) and contralateral (cL2), as well as the ipsilateral extensor-related ventral root (iL5). We found that low intensity stimuli delivered in an appropriate phase of locomotor activity triggered a phase advance activity in the ipsilateral ventral L2 root while high intensity stimulation induced a complex bilateral speeding effect lasting for seconds. This effect was characterized by an increase in burst frequency in all ventral roots with correct flexor-extensor and left-right alternation. The speeding effect was less pronounced as NMDA or DA concentrations were increased and could be abolished by bath application of the 2-methyl-6-(phenylethynyl)-pyridine (MPEP), antagonist of metabotropic glutamate receptor of subtype 5 (mGluR5), suggesting the critical role of mGluR5 for this speeding effect.
To reproduce and explain the experimental results of the dorsal root stimulation, we extended our earlier computational bilateral model of the locomotor CPG by incorporating sensory inputs. In the model, the rhythm generator (RG) on each side consists of flexor and extensor half-centers mutually inhibiting each other. Coordination between the left and right RGs is provided by excitatory and inhibitory commissural pathways. Sensory inputs drive two types of dorsal horn relay neurons, with low and high activation thresholds, which affect the CPG. High-threshold relay neurons incorporate mGluR5 receptors. Activation of mGluR5 by high-intensity afferent stimulation provides a long-lasting depolarizing effect on the RG, inducing speeding. Our model suggests that higher DA or NMDA concentrations presynaptically block glutamate release from sensory afferents to the relay neurons, or occlude the glutamate effect, thus reducing the speeding effect. Simulating MPEP application in the model by blockade of the mGluR5 receptors prevents speeding.
Our model proposes mechanistic explanations for the experimentally observed effects of afferent stimulation and provides novel insights into the organization of the locomotor central pattern generator.

Disclosures: N.A. Shevtsova: None. **S.B. Dietz:** None. **J. Ausborn:** None. **R.M. Harris-Warrick:** None. **I.A. Rybak:** None.

Poster

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: CRSNG / NSERC Grant 312015

Title: Effects of thermal stimulation of the face on limb movements in *In vitro* preparations of newborn opossums, Monodelphis domestica

Authors: *E. CORRIVEAU-PARENTEAU, T. CABANA, J.-F. PFLIEGER;
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Abstract: Marsupials are born very immature and must attach to the mother's nipples in order to continue their development. The newborn grey short-tailed opossum crawls on the mother's belly, unaided by her, using alternate rhythmic movements of the forelimbs (FLs). These movements are generated by the spinal cord but cephalic sensory systems are needed to sustain crawling, to guide the newborn to the nipple and to induce the attachment to it. In this species, we have shown that electrical stimulation of the trigeminal ganglion, which is the main source of sensory innervation of the snout skin, readily induces FL movements in *in vitro* preparations of newborn. We have further shown that mechanical pressures on the snout skin induce FL movements. To determine if sensory modalities other than touch could influence FL movements of newborn opossums, we tested the effect of temperature on facial skin. 11 newborns were deeply anesthetized before being eviscerated. Each specimen was placed in a petri dish filled with oxygenated physiological solution. Following craniotomy and laminectomy, decerebration was done by sectioning rostral to the midbrain. The limbs and tail were left attached to the carcass. Under a microscope 0.3 cc of solution at variable temperature was ejected from a needle directed towards the face skin. 10 ejections at each 3 temperature ranges: cold (4-8°C), warm (room temperature, 21-25°C), or hot (35-45°C), were made on each specimen, with a minimum interval of 3 min. between ejections. The FL responses were observed visually and described as non-locomotor-like: extensions of one FL or of both FLs without obvious interlimb coordination, or locomotor-like: alternate extensions of the left and right FLs. Ejections of the cold solution always induced FL movements (100%), whereas ejections of warm or hot solution induced movements 17% and 26% of the times, respectively. Locomotor-like responses accounted for 57% of the responses to cold, but of only 5% of the responses to the warm or hot temperature stimulations. After complete section of the spinal cord at the obex, only non-locomotor-like responses were obtained after cold stimulation, and in only 59% of the tests. The latter may be explained if the puff ejections of the liquid reached the neck or FL skin. Warm and hot temperatures were not tested in spinalized animals. These results show that temperature can

influence motor behaviors of newborn opossums and that cold is far more potent than warm and hot. We propose that exposure to colder temperature after birth may induce or sustain locomotion of the newborn until it reaches a nipple, thereafter other stimuli may inhibit this behavior.

Disclosures: E. Corriveau-Parenteau: None. **T. Cabana:** None. **J. Pflieger:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

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Program#/Poster#: 536.17/DDD1

Topic: E.07. Rhythmic Motor Pattern Generation

Support: ERC starting grant 'Optoloco' # 311673

Title: Investigation of neural circuits in the zebrafish hindbrain during active locomotion

Authors: *K. E. SEVERI, U. L. BOEHM, M. CHAVEZ, C. WYART;
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Abstract: Locomotion is a complex process relying on motor circuits in the spinal cord that integrate dynamic sensory feedback as well as descending commands from the brain. The hindbrain is a major control center to drive locomotor central pattern generators and perform sensory integration across modalities. In the larval zebrafish, activity patterns in the hindbrain apart from the reticulospinal neurons have been vastly underexplored. To characterize the hindbrain population driving motor output and integrating sensory signals, we perform *in vivo* two-photon calcium imaging in both paralyzed and actively swimming larval zebrafish expressing genetically-encoded calcium indicators under panneuronal drivers. By inducing locomotion with whole-field visual motion and simultaneously recording high-speed video of the moving tail during calcium imaging, we can link tail kinematics to neural signals. We used automated selection algorithms and registered active regions across individuals. To characterize the neural circuitry, we estimated the Granger causality between time series recorded from cells of interest. We also use specific markers to label subsets of the population and ask what role these neurons play during locomotion. These experiments contribute to a functional map of the hindbrain and by comparing active and paralyzed conditions, to explore activity associated with either locomotor drive or mechanosensory feedback mechanisms.

Disclosures: K.E. Severi: None. **U.L. Boehm:** None. **M. Chavez:** None. **C. Wyart:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

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Program#/Poster#: 536.18/DDD2

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Konrad-Adenauer-Stiftung

DFG grant Bu857/14

Title: Motor activity in the three major leg joints of the turning stick insect is modified in a joint- and context-specific way

Authors: *E. I. HAMMEL, A. BÜSCHGES, M. GRUHN;
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Abstract: Locomotion depends on neuronal activity, muscle contractions, and sensory feedback. The generation of straight walking has already been well studied and much is known about the neural activity underlying basic locomotor patterns in the single leg (Büschges 2005). However, we only begin to understand the neuronal mechanisms underlying behavioral flexibility, and the knowledge on local segmental activity during adaptive locomotion and the role of descending influences on it is scarce (Hellekes et al. 2012; Martin et al. 2015; Gruhn et al. 2016).
Here we investigate the neural mechanisms underlying curve walking in the stick insect (Carausius morosus). Turning of the tethered animals on the slippery surface was induced using an optical stimulus, and walking sequences were monitored by video and by EMG recordings of the *Flexor tibiae* muscle in both front legs. We studied the influence of optomotor induced turning on motor activity in all three major leg joints by recording extracellularly from leg nerves nl2 (containing protractor motoneurons (MNs)), nl5 (retractor MNs), C1 (levator MNs), C2 (depressor MNs), nl3 (extensor MNs), and branches of the nervus cruris (flexor MNs) of the deafferented meso- and metathoracic ganglia in a reduced preparation with all legs cut off except for the two front legs.
The motor activity of the three joints showed three types of responses: 1) a context-dependent change in activity in the subcoxal joint: meso- and metathoracic protractor MNs showed an increased activity during inside over outside turns (mesothorax (Ms) N = 4, metathorax (Mt) N = 12), while retractor MNs showed an increased activity during outside vs. inside turns (Ms N = 4, Mt N = 8). 2) a context-independent activity: the meso- and metathoracic levator and depressor MNs mostly did not show changes depending on the turning direction. The levator MN activity increased as soon as the front legs started stepping in either direction (Ms N = 8, Mt N = 6), whereas depressor MN activity ceased (Ms N =4, Mt N = 6). 3) no characteristic pattern for either inside or outside turns: extensor (Ms N = 8, Mt N = 9) and flexor (Ms N = 13, Mt N = 12) MNs showed either increase or decrease in activity upon inside or outside turning with high

variability in neuronal activity, without an obvious context-dependent pattern.
Our results indicate that changes in meso- and metathoracic motor activity during curve walking of the front legs are not thorax-segment specific but occur on the level of each leg joint. Further studies will clarify in how far the activity of meso- and metathoracic central pattern generators are affected by descending influence from curve walking front legs.

Disclosures: **E.I. Hammel:** None. **A. Büschges:** None. **M. Gruhn:** None.

Poster

536. Rhythmic Motor Patterns: Control Mechanisms

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Program#/Poster#: 536.19/DDD3

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Ministry of Education, Culture, Sports, Scienceand Technology of Japan (#24390431 to M.I.)

Title: Role of capsaicin sensitive nerves in initiation of swallows evoked by carbonated water stimulation in rats

Authors: ***K. TSUJI**, T. SUZUKI, S. SAKAI, J. MAGARA, T. TSUJIMURA, M. INOUE; Niigata Univ. Grad. Sch. of Med. and Dent. Sci., Niigata City, Japan

Abstract: Purpose: Capsaicin is known as a therapeutic drug which improves swallowing function and prevents aspiration pneumonia. However, a role of capsaicin-sensitive receptors/fibers on naturally evoked swallows has not yet been clarified precisely. The aim of the present study was to investigate effects of capsaicin-sensitive laryngeal nerves on swallowing evoked by mechanical and chemical stimulation. Materials and Methods: Urethane-anesthetized male Sprague Dawley rats were used. Swallowing reflex was identified by the electromyographic burst of suprahyoid and thyrohyoid muscles. Following topical application of capsaicin alone or together with QX-314 (QX-314/capsaicin) to the vocal folds, the number of swallows by capsaicin application to the vocal folds was measured at 5, 30 and 60 min after initial procedure. Effect of topical application of QX-314/capsaicin on the following swallows evoked by carbonated water stimulation and von Frey mechanical stimulation was also evaluated at 5 min. Results: While swallow initiation did not significantly change among the time points in capsaicin alone model, it was significantly suppressed in QX-314/capsaicin model at 5 min and was back in a time dependent manner. Topical application of QX-314/capsaicin inhibited swallows evoked by carbonated water stimulation. This was not the case of those evoked by

mechanical stimulation. Conclusion: Capsaicin sensitive nerves play an important role in initiation of swallows evoked by carbonated water stimulation.

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Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

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Topic: E.10. Motor Neurons and Muscle

Support: National Health and Medical Research Council of Australia

University of New South Wales International Postgraduate Research Scholarship

Neuroscience Research Australia Supplementary Scholarship

Title: Spinal changes after one session of isometric training of the elbow flexors are influenced by forearm posture

Authors: *J. NUZZO[1], B. BARRY[2], S. GANDEVIA[1], J. TAYLOR[1];
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Abstract: Strength training is motor training that involves repetitive high-force muscle contractions. After one session of isometric strength training of the elbow flexors, cervicomedullary motor evoked potentials (CMEPs) in biceps brachii increase in size for up to 25 minutes (Nuzzo et al. Med Sci Sports Exerc 2016). Thus, acute strength training can increase corticospinal excitability at a subcortical level. In *Experiment 1*, we examined if these spinal changes also occur with isometric training at low forces. Ten subjects completed low-force training (12 sets of 8 isometric contractions, 15% peak force) of the right elbow flexors in one session, and high-force training (12 sets of 8 isometric contractions, 75% peak force) in another. Training was performed with the forearm supinated and vertical, and the elbow and shoulder flexed to 90 degrees. Before and after training, electrical stimulation of corticospinal tract axons at the cervicomedullary junction elicited CMEPs in biceps brachii. Unexpectedly, biceps CMEPs were unchanged after both low- and high-force training. To determine if the lack of change in CMEPs after high-force training was due to the posture of the forearm during training, a second experiment was conducted. In *Experiment 2*, 14 subjects completed one session of high-force training with the forearm pronated rather than supinated. All other aspects of posture and the

training were unchanged. Biceps CMEPs were facilitated for up to 25 minutes after training. The increase in biceps CMEPs after high-force training in *Experiment 2* (51 ± 54.7%; mean ± SD) was greater than in *Experiment 1* (-0.5 ± 35.4%, $t = 2.603$, $p = 0.016$). Thus, spinal level changes (i.e., increased motoneuron excitability and/or increased efficacy of corticospinal-motoneuronal synapses) occur after one session of isometric strength training of the elbow flexors. However, these effects depend on the posture of the forearm during training. The mechanisms are unknown, but could include modifications in reflex paths and corticospinal transmission.

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Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

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Program#/Poster#: 537.02/DDD5

Topic: E.10. Motor Neurons and Muscle

Support: Ochsner Clinic Foundation

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Title: Transforming growth factor growth-supportive effect on delayed nerve repair is mediated via a non-SMAD signaling pathway

Authors: *D. NGUYEN[1], M. BEAVERS[2], L. ALLULLI[2], I. IWUCHUKWU[3], W. SULAIMAN[4];
[1]Neurosurg., Ochsner Hlth. Syst., New Orleans, LA; [3]Neurol., [4]Neurosurg., [2]Ochsner Med. Ctr., New Orleans, LA

Abstract: Background and Objective: Our previous works have demonstrated the mitogenic effects of transforming growth factor -beta (TGF-β1) and forskolin on Schwann cells reactivation and axonal regeneration in delayed repair nerve. In this study we examine the components of the TGF-β/Smad signaling pathway involved in modulating the mitogenic effects. **Methods**: Adult female SD rats were used for chronic tibial nerve injury and delayed repair. After 2 months, the transected nerve was repaired and treated with forskolin (0.5 uM), TGF-β1 (1ng/mL) plus forskolin, or saline-treated. After 6 weeks, the proximal, site of injury-repair, and distal nerve stump were harvested and frozen. Total RNA was prepared for real-time RT-PCR using custom TGF-β signaling PrimePCR arrays. The expression of Smad2, Smad3, Smad4, Smad7, transforming factor beta receptor type 1 (TBRI) and type 2 (TBRII) were analyzed. A cycle

threshold (Ct) of less than 34 was used as a cut-off for reliability. Data were normalized to GAPDH and fold change relative to forskolin- treated were determined. **Results**: All SMADs were detectable in all groups except for SMAD-3 and SMAD-7 but those detected were at very low levels. In the TGF-β1 /forskolin-treated group, expression of Smad2 and Smad 4 were decreased in both repair sites and distal nerve stumps but more so at the site of repair. In the forskolin-treated group, Smad2 was expressed at both locations and at 15-fold higher than the corresponding TGF-β1 /forskolin-treated nerves.The TBRI was expressed in both treatment groups, and decreased 7.3-fold and 4.3-fold i*n* the distal and site of repair of TGF-β1 /forskolin-treated nerves, respectively. The TBRII was expressed only at the site of repair and decreased 21-fold in TGF-β1 /forskolin-treated nerve stumps. Expression of TGF- β1 decreased 67-fold and 26-fold in the distal and site of repair respectively. **Conclusion**: TGF-β1/Smad signaling components were downregulated with TGF-β1 /forskolin treatment suggesting alternative pathways are involved in maintaining Schwann cells in a reactivated state necessary for axonal regeneration.

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Poster

537. Motor Neurons: Activity, Sensory, and Central Control

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Program#/Poster#: 537.03/DDD6

Topic: E.10. Motor Neurons and Muscle

Support: Yamagishi Project

Title: Hip joint movement modulates the neural plasticity in reciprocal Ia inhibition induced with patterned electrical stimulation

Authors: *Y. UENO[1], J. USHIYAMA[2,3];
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Abstract: BACKGROUND: Stroke patients often lose the function of Reciprocal Ia Inhibition (RI) from the tibialis anterior to the soleus, which is a key factor for smooth heel contact in walking. It was previously reported that Patterned Electrical Stimulation (PES) to the common peroneal nerve for 20-30 min temporarily enhanced the RI. In this study, we investigated whether PES combined with passive flexion/extension movement of the hip joint modulates RI. METHODS: Seven healthy young individuals voluntarily participated in this study. Three

stimulation protocols were assessed as follows: 1) PES (10 pulses at 100 Hz every 1.5sec: 900-1000 trains) was applied during non-weight standing (CON); 2) PES was applied at the phase of hip flexion during passive hip movements with no loads (FLX); 3) PES was applied at the phase of hip extension during passive hip movements with no loads (EXT). Passive hip movements were reciprocally induced, by examiners manually moving the handles of a rehabilitation device (EasyStand Glider^{TM}, Altimate Medical Inc., Minnesota, USA). The strength of RI was measured by using conditioning-test paradigms of soleus H-reflex. As for all participants, RI assessments were performed at before, 0 min, and 25 min after the three PES interventions mentioned above. RESULTS: The strength of RI was significantly increased in EXT (before, 5.17 ± 2.60%; after, 17.83 ± 4.15%; $p < 0.01$). In CON, RI was increased to some extent, but the effect was no significant (before, 6.97 ± 5.81%; after, 11.53 ± 7.45; $p = 0.49$).On the other hand, no change of RI was observed in FLX (before, 8.79 ± 6.76%; after, 8.89 ± 13.58%; $p = 1.00$). Additionally, from assessment at 25 min in all paradigms, we confirmed the strength of RI dropping back to the initial level. CONCLUSION: These results indicate that the effect of PES on RI can be enhanced by adding passive hip joint movements which induce Ia afferent activity of hip flexors. Such combination of nerve stimulation and movement may help future development of new rehabilitation methods for post-stroke patients.

Disclosures: Y. Ueno: None. **J. Ushiyama:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

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Program#/Poster#: 537.04/DDD7

Topic: E.10. Motor Neurons and Muscle

Support: MED-EL Austria 404-781-7042

Title: Stimulation of a denervated laryngeal muscle with low frequency promotes selective reinnervation and restores function

Authors: *Y. LI, S. HUANG, D. ZEALEAR;
Hearing and Speech Sci., Vanderbilt Univ. Med. Center/Oto, Nashville, TN

Abstract: Previously, electrical stimulation of a denervated canine laryngeal muscle was shown to promote reinnervation by original over foreign motoneurons. An implantable nerve stimulation-EMG system was used to index the appropriateness of reinnervation of the vocal fold abductor (posterior cricoarytenoid, PCA) muscle by inspiratory versus foreign reflex glottis closure (RGC) motoneurons following recurrent laryngeal nerve section and repair. In the

present study in 11 canines, a clinical model was used, where both nerves were sectioned and ventilation compromised due to loss of abduction. The EMG system and a pulse generator were implanted, the latter for electrical conditioning of PCA muscles. After nerve section, animals were randomly assigned to four groups to assess the effect of different muscle stimulus paradigms on reinnervation quality and degree of functional recovery: 1)40 pps train, 2)20 pps train 3)10 pps train and 4)control-no stimulation. One msec pulses were applied with 4 sec on/4 sec off duty cycle during the post neurorrhaphy regeneration period. In bimonthly sessions, spontaneous vocal fold movement was measured endoscopically during induced hypercapnea in the anesthetized animal. Rectified integrated EMG potentials were recorded from abductor muscles and adductor (thyroarytenoid, TA) muscles. Recordings were obtained during hypercapnic respiration to index reinnervation by inspiratory motoneurons, and during superior laryngeal nerve stimulation to index reinnervation by RGC motoneurons. Exercise tolerance was measured on a treadmill in the awake animal using pulse oximetry. Results demonstrated that all stimulated groups tended to have better results (i.e. higher rank order) in all outcome measures compared to the non-stimulated control group. The rank order stayed fairly consistent across all outcome measures: 10Hz > 20Hz > 40Hz > non-stimulated. It would appear that electrical stimulation of the denervated PCA muscle inhibited synkinetic reinnervation by RGC motoneurons and promoted selective reinnervation by its original inspiratory motoneurons. In addition, stimulation with low frequency, characteristic of the intrinsic activity of PCA inspiratory motoneurons, was more effective than high frequency in inducing correct reinnervation and improving functional recovery.

Disclosures: Y. Li: None. **S. Huang:** None. **D. Zealear:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 537.05/DDD8

Topic: E.10. Motor Neurons and Muscle

Support: Fondation Cotrel

NSERC

Title: Intermuscular coherence is altered in adolescent scoliosis patients

Authors: *M. SIMONEAU[1], C. BLUTEAU[1], J.-P. PIALASSE[1], J.-S. BLOUIN[2];
[1]Laval Univ., Quebec, QC, Canada; [2]Sch. of kinesiology, The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Adolescent idiopathic scoliosis (AIS) is a three dimensional deformation of the spine. AIS is often associated with vestibulomotor deficits and it has been proposed that an asymmetric descending vestibulospinal activity would cause an asymmetric motor drive to the torso muscles. Such asymmetrical muscles activity, during bone development, may lead to a spine deformation (Lambert et al., 2012). Although this hypothesis is attractive, a dysfunction of other descending pathways could also induce asymmetric motor drive to the torso muscles. The aim of this study was to compare the function of the descending corticospinal pathway between AIS patients (n=13, 11 girls, mean age = 13.8 ± 1.8 years) and healthy adolescents (n=12, 11 girls, mean age = 13.7 ± 2.0 years). The function of the corticospinal tract was assessed through intermuscular coherence between paraspinal muscles while participants performed isometric back extension at two different force levels (i.e., 15% or 30% of maximal strength). Each trial lasted 16 s and there were 15 trials per condition. Back extension strength was measured using a load cell located 0.1 m below the 7^{th} cervical vertebra. Right and left paraspinal muscle activity was recorded using surface electromyography at a sampling frequency of 1000 Hz. For each condition, coherence was estimated with concatenated data for each trial within each participant and concatenated pooled data for each trial across all participants. Significant differences between groups for the pooled coherence were determined with the difference of coherence test. Significant coherence between 8 and 12 Hz and 15 and 50 Hz was observed at both isometric force amplitudes. For the control group, 11 out of 12 participants demonstrated significant coherence for both frequency bands. For the AIS group, 8 out 13 participants exhibited significant coherence for the 15 to 35 Hz frequency band while only 2 participants showed significant coherence for the 8 to 12 Hz frequency brand. Results of the difference of coherence test revealed that regardless of the isometric force amplitude, AIS patients showed less coherence between the 8 to 12 Hz and 15 to 35 Hz frequency bands compared to controls. These results suggest a dysfunction of the descending corticospinal tract or the sensorimotor network involved in torso muscle activation in AIS patients.

Disclosures: **M. Simoneau:** None. **C. Bluteau:** None. **J. Pialasse:** None. **J. Blouin:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

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Program#/Poster#: 537.06/DDD9

Topic: E.10. Motor Neurons and Muscle

Title: b - Learning of mindfulness, studying body and brain unifying science through conscious trunk regulation using tactile information

Authors: *Y. ATOMI[1], R. TUZUKI[1], T. ATOMI[2], K. TANAKA[2], A. ATOMI[1], M. SHIMIZU[1], E. FUJITA[1], K. HASEGAWA[3];
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Abstract: We propose b-leaning, which connects body and brain to recreate human system. We have been presented in this meeting about dual instability of adhesive cells consisting of multicellular organism and human body bipedalism connecting by extracellular matrix (ECM) associating with voluntary movement and passive tension development under the gravity. We need integrating body-mind bi-directionality to control posture, especially slow voluntary movement like Noh and Tai-chi-chuan, which have been developed in Asia. Previously we reported that using analysis of ultrasonic images of abdomen muscles contractions during special standing performed in Tai-chi-chan using video images. Investigation of the neural substrate underlying whole body instability, based on the self-recognition paradigm revealed significant activity in the regions of the right parieto-insular vestibular cortex, inferior frontal junction, posterior insula and parabrachial nucleus (Atomi T et al., 2014). On the other hand, we have also reported the importance of tactile information using special wear to balance and normalize posture during standing, sitting and walking using 3D-motion analysis system (2015SfN). In the present study, we report the usefulness of one-week practices of voluntary conscious trunk exercises performed with touching by own hands, feeling the tactile information, and developing tension of own abdominal muscles at “the” area performed under recumbent position and scanning over whole ventral side of the body for 1~5 minutes per day. The effects were measured by physical performances like sit-up and jumping side to side, standing posture by photograph and questionnaire. Especially sit-up performance, standing posture and motivation to do active life significantly improved after one-week practice. Sit-up exercise is a special index showing good connection of whole body and brain according to Murata et al. (2010). It is very important for us to understand dual knowledge about not only own real human life system consisting of cells and ECM that are secreted by the cells, and responds well to mechanical stresses to increase during own physical exercise, and that works with activity-dependent survival rule, but also conscious movement bridging body and mind to control instable own body and conscious mind. Tactile information is essential for accepting own real system of body and imaginary brain systems creating human mind. Craig reviewed the neuroanatomical evidence for a progression of integrative representations of affective feelings from the body in the bilateral anterior insular cortex, or “the sentient self.”

Disclosures: Y. Atomi: None. **R. Tuzuki:** None. **T. Atomi:** None. **K. Tanaka:** None. **A. Atomi:** None. **M. Shimizu:** None. **E. Fujita:** None. **K. Hasegawa:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 537.07/DDD10

Topic: E.10. Motor Neurons and Muscle

Title: Protective role of monoamine oxidase B inhibitor on degenerating spinal neurons and its effects on nerve regeneration and functional recovery in sciatic nerve crush injury model

Authors: *H. M. ALSHIMALI[1], N. M. KAYALI[2], A. ALSALEM[2], W. M. RENNO[2], M. S. RAO[2], K. M. KHAN[2];
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Abstract: Monoamine oxidase (MAO) is a flavin adenine dinucleotide (FAD) containing an enzyme which catalyzes the oxidation of amines. MAO-B is proposed to play an important role in the pathogenesis of neurodegeneration through the production of reactive oxygen species (ROS) and neurotoxins. The present study was designed to outline the effects of the MAO-B inhibitor on sciatic nerve regeneration, neuroprotection of spinal neurons and sensory-motor functional recovery in the sciatic nerve crush injury model. Male Wistar rats (4-month-old) were assigned to i) Naïve (N), ii) Sham (S), iii) Sciatic nerve injured and treated with saline (I+saline) and iv) Sciatic nerve injured and treated with MAO-B inhibitor (I+MAO-B-I) groups (n=10/ group). In group iii and iv, the injury was produced by crushing the sciatic nerve followed by treatment with saline or MAO-B-I (2.5 mg/kg) for 10 days. Behavioral tests were conducted from week 1 to week 7. At the end of the study sciatic nerve and lumbar spinal cord were studied by immunohistochemistry, light and electron microscopy. Data were analyzed with one-way ANOVA followed by Bonferroni's multiple comparison tests. I+MAO-B-I treatment showed significant improvement in sensory and motor tests (Hoping reflex, hot plate test, tail flick test, extensor postural thrust, foot position, toe spread test, mechanical hyperalgesia test) compared to I+saline group ($p<0.05$ - 0.001). The morphological study showed a significantly increased number of nerve fibers in sciatic nerve ($p<0.05$), with better myelination pattern in I+MAO-B-I treated group compared to I+saline group. Spinal cord ventral horn showed a significant increase in the number of NeuN-immunoreactive neurons in the I+MAO-B-I treated group compared to I+saline group ($p<0.01$). These results show that MAO-B-I has a significant potential for protecting the degenerating spinal cord neurons and enhancing the regeneration of injured sciatic nerve following crush injury.

Disclosures: H.M. Alshimali: None. **N.M. Kayali:** None. **A. AlSalem:** None. **W.M. Renno:** None. **M.S. Rao:** None. **K.M. Khan:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

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Program#/Poster#: 537.08/DDD11

Topic: E.10. Motor Neurons and Muscle

Support: American Heart Association

Title: Quantification of a single score (1+) in the modified ashworth scale (MAS), a clinical assessment of spasticity quantification of the modified ashworth score, a clinical assessment of spasticity <!--[endif]-->

Authors: *N. L. SURESH[1], J. EWOLDT[2], E. ROTH[1];
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Abstract: The Modified Ashworth Scale (MAS) is an assessment that is often used by clinicians to grade spasticity in the affected limbs of stroke survivors. The MAS scale is a function of whether the clinician perceives a resistance to stretch and/or a 'catch' during a passive joint rotation. The qualitative nature of the assessment in combination with the low resolution of the scale could result in varied grouping of spastic patients, even for a single score. The objective of this pilot study was to develop a method for the quantification of the MAS, which could provide greater resolution and could eventually guide better informed therapeutic interventions. The MAS assessment for four stroke survivors with the same clinical MAS score of 1+ was performed by a clinician and quantified using signals from surface electromyography (EMG) and an electrogoniometer during passive joint rotation of the affected and contralateral upper limbs of stroke survivors. The findings from this study show a varied set of signals across four stroke survivors, all graded at 1+. The quantification provides insight as to the mechanisms underlying the passive resistance.

Disclosures: **N.L. Suresh:** None. **J. Ewoldt:** None. **E. Roth:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

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Topic: E.10. Motor Neurons and Muscle

Support: CNPq - Brazil Grant 205440/2014-2

Wellcome Trust Grant 101002/Z/13/Z

NIH Grant R01NS076589

NIH Grant R01NS090622

Title: A new method for assessing human upper limb Ib function in health and disease

Authors: *S. A. AGUIAR[1], S. CHOUDHURY[2], H. KUMAR[2], M. A. PEREZ[3], S. N. BAKER[1]; [1]Inst. of Neurosci., Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [2]Inst. of Neurosciences, Kolkata, Kolkata, India; [3]Dept. of Neurolog. Surgery - The Miami Project to Cure Paralysis, Univ. of Miami, Miami, FL

Abstract: Non-invasive assessment of spinal circuitry in human upper limb is important experimentally and clinically; however, methods are limited. We propose a new protocol in which we conditioned the flexor carpi radialis (FCR) H-reflex, evoked by median nerve stimulation, by previous extensor carpi radialis (ECR) stimulation - three times motor threshold (MT) -, at 18 inter-stimulus intervals (ISIs). Results showed significant facilitation at 30ms ISI and inhibition at 70ms ISI. We hypothesized the facilitation was caused by the action of Ib afferents from Golgi tendon organs in ECR, activated by the twitch that follows stimulation at such intensity. Our aim was to test this hypothesis and investigate this effect in disease. Several human experiments were carried out, which showed that: (i) the first peak in force production after the ECR shock starts in less than 30ms, meaning that the timing of ECR Ib activation was consistent with the hypothesis; (ii) stimulation given to ECR at MT did not cause facilitation, showing that the ECR twitch was necessary for the effect to occur; (iii) taps applied to ECR tendon instead of electrical stimulation produced a similar curve with the facilitation shifted around 15ms earlier; and the facilitation seemed to remain both (iv) when the protocol was applied with the wrist held in a flexed position, excluding the possibility that the effect was caused by afferents from FCR which was disengaged in this position, (v) as well as after vibration of ECR tendon for 25 minutes, which increases the threshold for electrical activation of Ia afferents but does not change the threshold for Ib activation. The main protocol was also tested in three anesthetized rhesus monkeys, with ECR dissected. Results showed a similar curve for monkeys, with the facilitation of the FCR response observed at 30ms ISI. Corroborating our hypothesis, the effect disappeared after cutting the ECR tendon, which meant that the muscle twitch did not pull on the tendon. Finally, we tested the main protocol in stroke and spinal cord injury human patients. Stroke patients presented a similar curve compared to healthy adults for intervals up to 30ms but the facilitation remained for intervals up to 60ms, while the inhibition was on average absent. Spinal cord injury patients showed no significant facilitation or inhibition in group average analysis. Our results provide evidence for a Ib pathway acting on the facilitation of an antagonist muscle in the forearm, which changes in disease. This experimental protocol could be used to assess the function of Ib pathways in human upper limb, since methods for such assessment are currently limited.

Disclosures: **S.A. Aguiar:** None. **S. Choudhury:** None. **H. Kumar:** None. **M.A. Perez:** None. **S.N. Baker:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

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Program#/Poster#: 537.10/DDD13

Topic: E.10. Motor Neurons and Muscle

Support: NIH K12HD055931

University of Iowa Carver College of Medicine and Dept. of Physical Therapy and Rehabilitation Science

Title: A novel method for cortical mapping of proximal and distal upper limb muscles using transcranial magnetic stimulation

Authors: *S. L. DEJONG[1], L. A. WERNER[1], W. G. DARLING[2], R. K. SHIELDS[1]; [1]Dept. of Physical Therapy and Rehabil. Sci., [2]Dept. of Hlth. and Human Physiol., Univ. of Iowa, Iowa City, IA

Abstract: Transcranial magnetic stimulation (TMS) is a powerful tool that can be used to probe connectivity between cortical motor neurons and upper limb muscles. The most frequently utilized TMS methods require time-consuming procedures to determine each muscle's optimal stimulation site and motor threshold (MT), followed by stimulation of surrounding sites at a percentage of MT to determine the muscle's cortical representation. Most prior studies have focused on one or two muscles of the hand or wrist, which typically have low MTs and large cortical representations. Although many research questions can be addressed using maps of one or two muscles to represent the upper limb, more comprehensive cortical mapping may allow further examination of cortical reorganization as it relates to motor recovery in humans. The purpose of this study was to demonstrate feasibility of an expanded method of TMS cortical mapping using a novel procedure. Ten healthy right-handed adults participated. An anatomical brain MRI was obtained for neuronavigation. A grid of stimulation sites was arranged over the left precentral gyrus and surrounding area. The site in the grid that was closest to the hand knob was used to identify the lowest stimulus intensity necessary to elicit motor evoked potentials (MEPs) ≥50 μV peak-to-peak in the first dorsal interosseous in ≥ 5 of 10 trials. Each site in the grid was stimulated 5 times at each of 3-5 stimulus intensities in increments of 10% of maximum stimulator output. MEPs were simultaneously recorded from nine proximal and distal muscles of the right upper limb. Each TMS stimulus was a single monophasic pulse, 70 μs, delivered

perpendicular to the precentral gyrus using a 75 mm butterfly coil. The order of stimulation sites, intensities, and inter-stimulus interval durations (4-10 s) were randomized. For each muscle, MEP amplitudes were expressed as a percentage of maximal EMG, cortical map volumes were calculated, and recruitment curve slopes were determined for sites where MEPs were elicited using 3 or more stimulus intensities. Results demonstrate feasibility of mapping multiple upper limb muscles simultaneously within a single session. While distal muscles showed greater map volumes as expected, maps of proximal muscles were also produced, with MEPs that typically exceeded 20% of maximal EMG. In future studies, this method may be a useful tool to probe connectivity after neural injury such as stroke, to predict potential for recovery, and to quantify changes in cortical organization.

Disclosures: S.L. DeJong: None. **L.A. Werner:** None. **W.G. Darling:** None. **R.K. Shields:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

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Program#/Poster#: 537.11/DDD14

Topic: E.10. Motor Neurons and Muscle

Support: NCN 2013/09/B/NZ4/03306,

Statutory grant for the Nencki Institute

Title: Stimulation of low-threshold proprioceptive fibers increases density of glutamatergic and cholinergic boutons on the ankle extensor α-motoneurons in the adult rat

Authors: *O. GAJEWSKA-WOZNIAK, J. CZARKOWSKA-BAUCH, K. GRYCZ, M. SKUP;
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Abstract: In our previous study chronic stimulation of low-treshold muscle afferents (Ia) in the tibial nerve in awake rats caused clear increase of expression of neurotrophin NT3, in the spinal cord and soleus muscle, being a good predictor of synaptic plasticity in this spinal circuit (Gajewska et al., 2013). This paradigm of stimulation can reinforce NT3-dependent, glutamatergic Ia input to the extensor α-motoneurons (MNs) but also effect indirectly, through spinal interneurons, including cholinergic V0c group modulating activity of MNs via C-terminals. We aimed to examine the effectiveness of the same paradigm of stimulation, which caused an increase in NT-3, on morphology and density of both inputs to α-motoneurons (MNs)

of the lateral gastrocnemius (LG) muscle. Tibial nerve was stimulated unilaterally for 7 days with continuous bursts of three pulses in four 20 min sessions daily. The Hoffmann reflex recorded from the soleus muscle, LG synergist, allowed controlling low-threshold stimulation. LG MNs were identified with intramuscularly injected tracer. Glutamatergic Ia terminals and cholinergic C-terminals were detected immunohistochemically, using input-specific anti-VGLUT1 and anti-VAChT antibodies, respectively. The total population of synaptic terminals was identified with Synaptophysin immunolabeling. Quantitative analysis of terminals on LG MNs, captured with confocal microscopy, revealed that the number of VGLUT1 and of VAChT immunoreactive terminals contacting the soma increased after stimulation by 35% and by 20%, respectively, comparing to the sham-stimulated side. Analysis of frequency distribution of boutons, distinguishing arbitrarily 4 classes of terminals volumes, revealed that this enrichment occured in groups of smallest boutons (VGLUT1: 1-5 μm^3 VChAT: 5-10 μm^3) in expense of the larger boutons (volume > 10 μm^3). This combined data may indicate that 7 days of stimulation of Ia afferents is sufficient to cause a formation of new glutamatergic and cholinergic terminals, both characterized by their small volume at this stage. Moreover, an increase of labeling intensity in the larger cholinergic and glutamatergic terminals (>10 μm^3; $p<0.03$ and $p<0.23$, respectively) was observed. On the contrary, labeling intensity in the smallest boutons did not change, suggesting that newly formed boutons contain a smaller number of the vesicular transporter molecules. To conclude, one week of stimulation of proprioceptive Ia input to LG MNs is effective in enrichment of their direct glutamatergic but also indirect cholinergic innervation. Our findings confirm also that this input is adequate for activation of $V0_C$-cholinergic interneurons.

Disclosures: O. Gajewska-Wozniak: None. **J. Czarkowska-Bauch:** None. **K. Grycz:** None. **M. Skup:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

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Topic: E.10. Motor Neurons and Muscle

Support: Heart and Stroke Foundation (British Columbia and Yukon).

Heart and Stroke Foundation Doctoral Award

Canadian Institutes for health Research(CIHR) Grant #MOP-106651

Title: Unilateral wrist extension training after stroke improves function bilaterally

Authors: *Y. SUN[1,2,3,4], N. LEDWELL[5], L. BOYD[5], E. ZEHR[1,2,3,4,6];
[1]Rehabil. Neurosci. Laboratory, Univ. Victoria, Victoria, BC, Canada; [2]Ctr. for Biomed. Research, Univ. of Victoria, Victoria, BC, Canada; [3]Human Discovery Science, Intl. Collaboration on Repair Discovery (ICORD), Vancouver, BC, Canada; [4]Exercise Science, Physical & Hlth. Education, Univ. Victoria, Victoria, BC, Canada; [5]Brain Behaviour Laboratory, Univ. of British Columbia, Vancouver, BC, Canada; [6]Div. of Med. Sciences, Univ. of Victoria, Victoria, BC, Canada

Abstract: Stroke produces muscle weakness seen on both more (MA) and less affected (LA) sides. "Cross-education" describes the phenomenon that training one side of the body increases strength or motor skill in the same muscles on the untrained side. This can be applied to enhance muscle strength in the MA side and we found that 6 wks of dorsiflexion resistance training with the LA leg improved strength bilaterally in chronic stroke. Whether this can be observed in the arms was assessed with a 5-week unilateral wrist extension training intervention using the LA arm in 24 participants (> 6 m post-stroke, 65.6±6.7 y/o; 12 at UVIC, 12 at UBC). Maximal voluntary contraction (MVC) wrist extension force was measured with a 6-axis load cell using Cartesian coordinates (F_z = extension). Electromyography (EMG) of extensor (ECR) and flexor (FCR) carpi radialis (ECR), biceps (BB) and triceps brachii (TB) were recorded and the Fugl-Meyer and partial Wolf Motor Function Test were performed by the same physical therapist at each location. Reciprocal inhibition (RI) from wrist flexors to extensors, cutaneous reflexes evoked by median (MED) and superficial radial (SR) nerve stimulation were assessed in those at UVIC. Cortical silent period (CSP), short-interval intracortical inhibition (SICI), intracortical facilitation (ICF) and transcallosal inhibition (TCI) from transcranial magnetic stimulation were measured in participants at UBC. Twenty participants completed follow- up MVC extension force and EMG tests in both arms 5 wk after training.
F_z increased ~21% ($p<0.01$) in the LA arm and ~24% ($p=0.057$) in the untrained MA arm. Total force (F_{tot}) increased ~17% ($p<0.01$) and ~24% ($p<0.05$) in LA and MA respectively. Eighteen of 24 participants showed significant increases in F_z in their trained LA side and 11 showed significant increases in the untrained MA side. Improvement was also found in Fugl-Meyer motor function score ($p<0.01$). Significant correlations between muscle activation and size of RI were found only in the LA side before and after training. Correlation between muscle activation and SR cutaneous reflexes was significant bilaterally after training. LA CSP significantly decreased after training. Strength in 10 of 18 who showed strength increases in LA F_z, was retained, and 5 of 9 retained MA strength gains at follow up.
Results show LA training can facilitate wrist extension strength and function bilaterally with clinical transfer and neural plasticity. However, response variability between participants indicate that cross-education between arm muscles strength training might not as strong as in the legs in stroke participants.

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Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

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Program#/Poster#: 537.13/EEE2

Topic: E.10. Motor Neurons and Muscle

Support: a Grant-in-Aid from the Japan Society for the Promotion of Science Fellows (no.26-3962).

Title: Changes in spinal reflex excitability associated with motor imagery training

Authors: *S. KUBOTA[1,2], M. HIRANO[1,2], S. TANAKA[1], S. TANABE[3], K. FUNASE[1]; [1]Dept. of Human Sciences, Grad. Sch. of Integrate Arts and Sci., Hiroshima Univ., Higashi-hiroshima, Japan; [2]Res. Fellow of the Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Fac. of Rehabilitation, Sch. of Hlth. Sci., Fujita Hlth. Univ., Toyoake, Japan

Abstract: Objective: Motor imagery training has a significant impact on the functional alterations in brain regions. However, the effect of motor imagery training on the alternations in the spinal cord has not been clarified. The aim of this research is to investigate whether the motor imagery training can alter the spinal cord excitability. **Methods:** Ten healthy subjects participated in two experimental sessions (motor imagery training, no-training), conducted on 2 separate days, with an interval of 7 to 9 days between two consecutive sessions. The experiment procedure included a pre-training (motor execution), pre-test, motor imagery training/no-training, and post-test. In motor imagery training session, subjects imagined ankle dorsiflexion movement acording to visual guide that was used by motor execution period. In no-training session (control), subjects were instructed not to imagin any movement and was not given visual guide. The soleus H-reflex and the amount of Ia presynaptic inhibition (D1 inhibition) were assessed at pre-test and post-test period, which were measured under resting and motor imagery condition. **Results:** In motor imagery training session, the size of H-reflex decreased and the amount of D1 inhibition increased in 8 out of ten subjects. These changes were only observed in a motor imagery condition, but not in a resting condition. In control session, there were no changes observed in the size of H-reflex and the amount of D1 inhibition. **Conclusion:** Our results demonstrated that motor imagery training can alter not only the activity of brain, but also that of spinal cord. Previous studies have shown that motor imagery training improves motor performance in patients with central nervous disorders. Our findings provide new insight into the mechanisms underlying motor recovery associated with motor imagery training.

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Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

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Program#/Poster#: 537.14/EEE3

Topic: E.10. Motor Neurons and Muscle

Support: Lundbeck Foundation

Danish Medical Research Council

Jytte and Kaj Dahlboms Foundation

Title: Prolonged excitability and morphological changes following strenuous repetitive activity in mouse motor axons

Authors: S. ALVAREZ[1], C. KRARUP[1,2], *M. MOLDOVAN[1,2];
[1]Univ. of Copenhagen, Copenhagen, Denmark; [2]Dept. of Clin. Neurophysiol., Rigshospitalet, Copenhagen, Denmark

Abstract: During prolonged repetitive activity, the restoration of axonal ionic homeostasis lags behind the restoration of electrical properties. Most notably, the intra-axonal Na^+ accumulation due to influx through the voltage-gated Na^+ channels is counteracted by the activation of the energy-dependent Na^+/K^+ pumps leading to membrane hyperpolarization. We previously found that in mature mice, a strenuous repetitive electrical stimulation (RS) protocol (200 Hz for 3 hours) does not cause axonal degeneration. Nevertheless, even at 3 days after RS, the motor axon excitability remained changed. Given the very long timescale it was unlikely that these changes could be attributed to Na^+/K^+ hyperactivity. The aim of this study was to specifically investigate in adult mice (C57BL/6 background) the recovery of motor excitability up to 2 weeks after RS. Serial multiple measures of motor axon excitability of the tibial nerve at ankle were obtained by "threshold-tracking" of the evoked plantar compound muscle action potentials. To facilitate serial in vivo imaging using the Confocal Laser Endomicroscopy (Cellvizio Lab) we used mice expressing YFP fluorescent reporter under the mouse thymus cell antigen 1 - Thy1 promoter. At 1 week, the excitability on the RS side was undistinguishable from the contralateral side. At 3 days, however, the excitability was still reduced after RS, as indicated by the increased rheobase and larger than normal threshold changes during hyperpolarizing electrotonus. Interpretation of multiple excitability changes was carried out by optimizing the "Bostock" space-clamped myelinated axon model where the node and the internode are connected by a leak pathway referred to as the Barrett-Barrett conductance (GBB). We found that the prolonged excitability abnormalities after RS could largely be explained by changes in passive cable properties, most notably the increase in GBB. This was consistent with thinning of the internodal axon associated with altered paranodes quantified by automatic image analysis of optic cross-sections at the

stimulation site. Our data suggest that strenuous RS in mice leads to axonal thinning, possibly due the increased periaxonal space under the myelin, which leads to a prolonged impairment of motor axon excitability.

Disclosures: S. Alvarez: None. **C. Krarup:** None. **M. Moldovan:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 537.15/EEE4

Topic: E.10. Motor Neurons and Muscle

Title: Chemical Neuro Stimulation of the upper alimentary canal by TRPV1 and TRPA1 activation decreases muscle cramp frequency and severity

Authors: *G. SHORT[1], L. B. ROSEN[1], R. SUTHERLAND[1], J. LIU[1], J. M. CERMAK[1], G. MAIER[2], T. WESSEL[1];
[1]Flex Pharma Inc, Boston, MA; [2]MaierMetrics, Boston, MA

Abstract: Chemical Neuro Stimulation is the treatment of neurological disorders by using small molecules applied topically to sensory neurons to alter the behavior of distinct neural circuits within the central nervous system. We have devised one such approach whereby the co-activation of TRPV1 and TRPA1 ion channels in the upper alimentary canal decreases muscle cramp frequency and severity. Based upon evidence that α-motoneuron hyperexcitability is the underlying cause of cramps and spasticity, we hypothesized that TRP activation could provide sufficient excitatory sensory input via the solitary tract to modulate descending spinal pathway signaling to increase interneuronal inhibition and dampen motor neuron hyperexcitability. We have generated data that suggests that either an oral solution containing a mixture of naturally-derived TRP activators (TRP-Stim) or FLX-787, a synthesized single molecule TRPV1/TRPA1 co-activator, stimulate TRP ion channels in the mouth, oropharynx and esophagus in a local, topical fashion to inhibit muscle cramping. Efficacy studies using an electrically-induced cramp (EIC) model demonstrated that both TRP-Stim and FLX-787 significantly reduced cramp intensity by as much as 3-fold relative to inactive control ($p<0.01$). Moreover, the pharmacokinetic profile of FLX-787 could not account for its EIC efficacy, as no systemic exposure of the parent form of FLX-787 in human plasma was observed. In both animals and humans, FLX-787 was found to undergo rapid phase 2 metabolism, resulting in extensive conjugation 15 minutes after ingestion, predominantly to glucuronide and sulfate metabolites. Even at doses up to 250 mg/kg in rats, the conjugates of FLX-787 accounted for >90% of circulating drug. To understand if topical exposure to mucous membranes in the mouth,

oropharynx and esophagus mediates the TRP-Stim and FLX-787 effect (given the lack of systemic exposure to FLX-787), the TRP-Stim mixture was encapsulated in gelatin capsules. Ingestion of the encapsulated mixture afforded no EIC efficacy relative to vehicle control. These results suggest that the observed effect on electrically-induced muscle cramps does not depend on the systemic bioavailability of TRP activators but rather on topical exposure of sensory neurons and consequent neuronal signaling. Given that efficacy has also been observed in nocturnal leg cramp (NLC) subjects with TRP-Stim (cramp-free days, $p<0.01$), Chemical Neuro Stimulation may be a general approach to develop novel treatments for cramps, spasms and spasticity. Based upon these findings, we expect to initiate studies with a chemically-synthesized single molecule TRP activator in NLC, MS and ALS.

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Poster

537. Motor Neurons: Activity, Sensory, and Central Control

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Topic: E.10. Motor Neurons and Muscle

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Title: A computational motor neuron pool model for the development of motor decoder algorithms for prosthetic control

Authors: *J. M. ALLEN[1], S. M. ELBASIOUNY[2];
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Abstract: Amputees have some intact neural circuits that are no longer connected to a muscle. Accordingly, the firing rates of their residual motor nerves can still code the characteristics of the intended movement. If decoded successfully, these signals could serve as the control for driving their prosthetic device. This study aims to develop motor decoder algorithms that predict the motor intent from motor unit firing rates for prosthetic control. To accomplish this goal, a high-fidelity computational model of a spinal motor neuron pool innervating the gastrocnemius muscle was developed. The model includes detailed representations of the 3 types of α-MNs (S, FR, and FF) which are built upon experimentally-obtained 3-dimensional anatomical data. The cells are represented in their respective proportions within the motor unit pool for this muscle. The passive cell membrane and active conductance data were set to match experimental data from cat α-MNs.
Initially, cells were stimulated using current injection, and optimized to behave comparably to in vitro electrophysiological recordings obtained from similar cells under a current clamp protocol. Later, uniform systems of sensory and motor synapses were applied across the dendritic arbor of the model cells and used to generate an output in the form of pool and individual cell firing data. Firing data from the model was able to be encoded to simulate production of muscle force and EMG. Results to date also have implications for development of a direct conversion of descending synaptic input to muscle force.

Disclosures: J.M. Allen: None. **S.M. Elbasiouny:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 537.17/EEE6

Topic: E.10. Motor Neurons and Muscle

Title: Effect of hyperbaric oxygen therapy on h-reflex and grip strength

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Abstract: Objectives: Hyperbaric Oxygen Therapy (HBOT) has been used for a number of pathologic conditions affecting neuromuscular and musculoskeletal conditions. It might promote cellular metabolism of different tissue systems such as promoting wound healing. It showed to be useful in decompression sickness and possibly children with Autism. However, no didactic studies have been carried out to evaluate the effects of HBOT on the physiologic and mechanical processes in human. The purpose of this study is to evaluate the effect of HBOT on H-reflexes and grip strength before and after 15 min. cycling, in healthy subjects. **Methods:** 16 healthy subjects (10 males and 6 females) with the age range of 23-69 signed a consent form. Soleus H-reflex was tested in both lower limbs during lying and standing before and after 60 min. session of HBOT at 3 PSI and after 15 min cycling. H-reflexes were recorded from the soleus muscle after stimulation of the tibial nerve at the popliteal fossa (1 msec. 0.2 PPS at H-max.) using surface electrodes and Cadwell EMG unit. Dependent parameters included the peak to peak amplitude of the H-reflex, H-latency, and maximum grip strength. Maximum grip strength was tested for the both hands before and after HBOT. Five H-traces were averaged for each trial and descriptive statistics were completed.
Results showed a significant increase in the peak to peak amplitude of the H-reflex after the HBOT ranging from 3.4-35%, which was more pronounced during lying than standing. This was associated with a mild increase in the grip strength ranging from 1.7-21.4%. Cycling appears to increase the H-amplitude further augmenting the HBOT effect on the motor neuron excitability. No change in the H-latency was recorded. These results indicate that HBOT increases the sensitivity of the muscle spindle afferents and motor neurons excitability resulting in the recorded increase of the grip strength. Cycling post HBOT appears to augment such effect due to the increased circulatory effect of the oxygenated blood in the neuromuscular system.
Discussion and conclusions: These results indicate that HBOT induces systemic effects on the neuromuscular and musculoskeletal systems of healthy subjects. Accordingly, HBOT could be useful as a rehabilitation procedure in patients with neuromuscular and musculoskeletal disorders. It is therefore recommended to use HBOT as an adjunct for rehabilitation procedures in patients with musculoskeletal and neuromuscular disorders.

Disclosures: M.M. Sabbahi: None. **F. Ovak Bittar:** None. **M. Badghaish:** None. **A. Al Maliki:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

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Program#/Poster#: 537.18/EEE7

Topic: E.10. Motor Neurons and Muscle

Support: Fapdf n. 193001010/2015

CNPq (n.476830/2013-3)

Title: Changes in postural control and presynaptic inhibition after ischemic conditioning

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Abstract: A number of interventions improve the control of upright posture by alterations in spinal cord processes. Previous report showed improvement of balance after the remote (upper) limb ischemic conditioning (RLIC), but little is known about the underlying neurophysiological mechanisms. Presynaptic inhibition (PSI) of the afferent Ia terminal is an important mechanisms involved in the control of gain of the stretch reflex pathway. The aim of this work is to evaluate the contribution of PSI to a possible improvement of balance caused by RLCI of the lower limb. Five healthy, sedentary male subjects, aged between 18 and 32 years, were selected to participate. The PSI was induced by an electrical stimulus with 1 ms duration applied to the common peroneal nerve 100 ms before the delivery of the test stimulus to the tibial nerve (to elicit the H-reflex in the soleus muscle). Postural sway was assessed with the subject standing on a high density foam placed over a force platform. The balance test consisted of 6 attempts with 1 minute each and 1 minute of rest, with either eyes open or closed. The root mean square (RMS) and the mean velocity (MV) of the center of pressure were calculated. The RLIC was performed in the left thigh (after the electrophysiological and biomechanical experiments) and along the three days thereafter. On the fifth day the experiments were repeated without the RLIC. The protocol was divided into three phases. The Sham and Conditioning phases were conducted over five days each, in subsequent weeks. During the Sham phase, the RLCI was not entirely effective and was just applied to fool the participant. The same procedure was followed for the Conditioning phase with the ischemia causing full obstruction. The Follow-up phase consisted of one day with the electrophysiological and biomechanical experiments in the week following the Conditioning phase. It is expected that the RLIC improves the balance performance as reflected by the parameters RMS and MV. We hypothesized that changing in PSI values will reflect neuronal adaptations following RLCI, which might be involved in the improvement of balance performance. The preliminary results showed a slight increase in the variable VM after RLCI, which might reflect postural adjustments to increase postural stability in a challenging condition. A tendency to reduced PSI after conditioning was also noticed, but did not reach statistical significance (paired t-test). These results suggest that mechanisms of PSI within the spinal cord might reflect neuronal adaptations resulted from RLCI. Additional experiments using the same paradigm will be conducted to either confirm or refute this speculation.

Disclosures: R.A. Mezzarane: None. **I.C. Quadrado:** None. **R.B. Câncio:** None. **B.M. Silva:** None. **L.C. Vianna:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 537.19/EEE8

Topic: E.10. Motor Neurons and Muscle

Support: NSERC

Title: Changes in central and peripheral excitability during rest and a slight contraction of the elbow flexors following an intermittent submaximal isometric contraction protocol

Authors: *B. W. COLLINS[1], L. H. GALE[1], N. C. M. BUCKLE[2], M. R. MONKS[2], K. E. POWER[3,4], D. C. BUTTON[2,4];
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Abstract: The excitability of the neuromuscular system is history-dependent. The purpose of the current study was 1) to assess the effects of repeated, submaximal voluntary contractions of the elbow flexors on measures of central (i.e. corticospinal) and peripheral (i.e. muscle contractile properties) excitability, as well as maximal voluntary contraction (MVC) force production and 2) to determine if measures of central and peripheral excitability are state-dependent (i.e. rest versus active). Eight chronically resistance trained males completed two randomized experimental sessions (rest and 5% MVC). During each session, participants received three stimulation protocols: 1) transcranial magnetic stimulation (TMS) of the motor cortex to determine corticospinal excitability (CSE) of the biceps brachii, 2) electrical stimulation of the biceps brachii motor point to determine evoked contractile properties of the elbow flexors and 3) electrical stimulation of Erb's point to determine maximal muscle compound action potential (M_{max}). TMS-induced motor-evoked potentials (MEPs), potentiated twitch (PT), rate of force development (RFD), and M_{max} were recorded for 2 minutes prior to, immediately, and 5 minutes following a submaximal contraction protocol that consisted of five intermittent voluntary contractions at 50% of MVC. MVC of the elbow flexors was also recorded prior to and 7 minutes post-contraction protocol. During rest, MEP amplitudes increased (213 ± 52% and 158 ± 29% of maximal M wave) at 1 and 5s post-contraction protocol, respectively but did not differ at any other time points thereafter. PT and RFD increased immediately post-contraction protocol and remained elevated for two minutes (PT: range 127 to 147% increase; RFD: range 127 to 141% increase). Both PT and RFD did not differ between pre- and 5 minutes post-contraction protocol. During a 5% MVC contraction there were no changes in MEPs from pre- to immediately and 5 minutes post-contraction protocol. PT and RFD increased immediately post-contraction protocol and remained elevated for two minutes (PT: range 123 to 134% increase;

RFD: range 128 to 139% increase). Both PT and RFD did not differ between pre- and 5 minutes post-contraction protocol. There was no effect of the contraction protocol on MVC force. In addition, immediately post-contraction protocol changes in resting CSE were positively correlated with PT (r = 0.886) but not during the 5% MVC. In conclusion, the changes in central and peripheral excitability following a submaximal contraction protocol are state (rest versus 5% MVC) and time (immediate but not at 5 minutes) dependent.

Disclosures: B.W. Collins: None. **L.H. Gale:** None. **N.C.M. Buckle:** None. **M.R. Monks:** None. **K.E. Power:** None. **D.C. Button:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

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Program#/Poster#: 537.20/EEE9

Topic: E.10. Motor Neurons and Muscle

Support: DFG Pf 128/32-1

Title: The effect of hunger on skeletal muscle innervation by octopaminergic modulatory neurons in larval and adult fruit flies, *Drosophila melanogaster*.

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Abstract: Biogenic amines are important modulators of behavior in all organisms. In insects, Octopamine, the homologue to vertebrate norepinephrine, is associated with either associative learning, energy demanding motor behaviors or adaptive behaviors to stress, for example caused by hunger. It had been previously shown by Koon et al (*Nature 14:190-99,2011*) and Koon and Budnik (*J Neurosci 32:6312-22,2012*) in live-imaging experiments on selected muscle fibers that a 2hr period of hunger in larvae induces significant changes in the morphology and function of octopaminergic axonal type II terminals and, in addition, of the neuromuscular junction (type I terminals). In our study we tried to unravel the dynamics of these changes with respect to the octopaminergic innervation in both larval body wall and adult flight muscles by assessing the total innervation pattern of selected muscle fibers. Although the actual periods of hunger varied greatly between larvae (2 to 6 hours) and adults (6 to 18 hours), the results nevertheless show a common theme: Compared to fed control groups a 2h period of hunger in larvae and of 6h in adults caused a temporary decrease in axonal length and number of branches before in larvae a significant increase above the control level is observed. Such an increase above control levels is not observed in adults. In larvae this corresponds well to behavioral essays of increased larval

locomotion. In order to find out about adult flies, long term monitoring the motor activity of isolated flies revealed that hungry flies move significantly for longer distances, significantly for longer durations and they move significantly faster but the number of movement bouts is not increased. Fed flies tend to stay at the bottom of the vial whereas hungry flies are more distributed.

Disclosures: H. Pflueger: None. **T. Mathejczyk:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 537.21/EEE10

Topic: E.10. Motor Neurons and Muscle

Support: Heart and Stroke Foundation of Canada (BC & Yukon)

Title: The arms really can give the legs a helping hand - rhythmic arm cycling training improves walking and interlimb connectivity in chronic stroke

Authors: C. KAUPP[1,2,3,5], T. KLARNER[1,2,4,5], Y. SUN[1,2,4,5], N. ZAPOTOCZNY[1,4], H. CULLEN[1,2,4,5], T. S. BARSS[1,2,4,5], G. E. PEARCEY[1,2,3,5], *E. ZEHR[1,2,3,4,5];
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Abstract: Spinal networks regulating locomotion remain intact and partially accessible following stroke. These networks can be activated by a variety of rhythmic movements, including arm and leg cycling. Previous work has shown that activation of interlimb networks through arm and leg cycling improves walking in post-stroke individuals. This study aimed to reveal whether similar results could be achieved by training only the arms. Nineteen chronic stroke participants (> 6 months post infarct) were recruited to this 5-week (30 min @ 60rpm x 3 / wk) training study with a within-subject multiple baseline control. Rate of perceived exertion (RPE) as well as heart rate (HR) were monitored throughout the 5 weeks to ensure that the physiological cost did not exceed moderate. Resistance was added to the Sci-Fit Pro 2 ergometer only if the participant was able to maintain 60rpm without a large change in either RPE or HR. During post-tests, strength was assessed bilaterally via maximal voluntary isometric contractions of the legs (plantarflexion and dorsiflexion) and the hands via grip strength. Muscle activation during treadmill walking and rhythmic arm cycling was assessed in the more affected (MA) and less affected (LA) side via surface electromyography (EMG) in the following muscles; tibialis

anterior (TA), soleus (SOL), anterior deltoid (AD), biceps brachii (BB), triceps brachii (TB) and flexor carpi radialis (FCR). Changes to interlimb coupling during rhythmic movement were evaluated via cutaneous reflexes elicited by electrical stimulation of the superficial radial nerve (5x1.0ms trains @ 300Hz). Bilateral soleus stretch reflexes were elicited at rest and during 1Hz cycling. Clinical status was evaluated by a physiotherapist using a variety of tests, including the 6 Minute Walk (6MW), Berg Balance Scale (BBS), and Chedoke-McMaster Stroke Assessment (CMSA). Post training there was a significant increase in bilateral grip strength, as well as in force during MA plantarflexion. Several arm muscles were subject to small changes in reflex modulation during arm cycling. During treadmill walking, MA TA was significantly more active during swing phase, and less active during stance, as compared to baseline levels. Roughly half of participants showed significant changes to stretch reflex amplitudes, and these changes varied in a bidirectional manner. During clinical evaluations, walking speed and balance improved along with positive changes in CMSA ratings of the hand and foot. Taken together, these results further elucidate the need for incorporation of upper limb training in the functional rehabilitation of walking after neurotrauma.

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Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

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Program#/Poster#: 537.22/EEE11

Topic: E.10. Motor Neurons and Muscle

Support: CNPq (n. 476830/2013-3)

Title: Effects of remote limb isquemic conditioning on the recruitment of motor units and synaptic transmission

Authors: ***I. C. QUADRADO**[1], R. B. CÂNCIO[2], B. M. SILVA[3], L. C. VIANNA[2], R. A. MEZZARANE[2];
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Abstract: The mechanisms behind the effects of the ischemic conditioning (IC) are still a matter of debate. Studies have shown that sub-lethal ischemia generates a protective effect on tissues from a subsequent ischemic event. A recent work reported enhanced motor learning after an IC of the upper limb. However, the neurophysiological mechanisms responsible for these effects are

unknown. We hypothesized that spinal cord processes, such as patterns of motor unit recruitment and homossynaptic depression, might reflect the adaptations of the central nervous system following an IC of the lower limb. Five healthy and sedentary male subjects, between 18 and 32 years, participate in the present study. The recruitment curve (RC) of the right soleus H-reflex was built by changing the electrical stimulus intensity applied to the tibial nerve (TN). A sigmoid function was adjusted to H-reflex amplitude values in order to obtain parameters that reflect patterns of recruitment of the soleus motor units. The homosynaptic depression (HD) was estimated by delivering pairs of pulses to the right TN at 1 Hz. The amount HD was evaluated by the ratio of H-reflexes elicited by the first and second pulses delivered 1 second apart. The IC was performed in the left thigh. The procedures were divided into three parts: Sham, Conditioning and Follow-up. Both Sham and Conditioning experiments were conducted along five days each in subsequent weeks. On the first day of the Sham phase, both the RC containing 30 points and the HD level (average of 20 reflex responses) were obtained. After those protocols, the “Sham Ischemia” was performed on the same day and 3 days thereafter. In the fourth day the RC and HD was evaluated without IC. The same procedure was repeated in the following week for Conditioning, however, using a procedure for ischemia that induced a total block, i.e., five minutes of ischemia alternated with five minutes of rest. The ischemia procedure was repeated five times. The Follow-up consisted of experiments (without IC) performed in the third week to obtain the RC and the HD level. The preliminary results showed a general decrease in the parameters obtained from the recruitment curve ($p<0.05$; paired t-test), which means an overall reduction in reflex excitability for most of the recruited motor units of the soleus muscle. No apparent change was observed for HD. These results suggest that IC can induce plasticity at the spinal cord level in humans, but one mechanism of synaptic transmission (HD) seemed not to be affected.

Disclosures: **I.C. Quadrado:** None. **R.B. Câncio:** None. **B.M. Silva:** None. **L.C. Vianna:** None. **R.A. Mezzarane:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

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Topic: E.10. Motor Neurons and Muscle

Support: MEXT 26293397

MEXT 15K15687

Title: Serotonergic modulation of glutamate-evoked responses through 5-HT_{2A} receptors in the dendrites of rat jaw-closing motoneurons

Authors: M. DANTSUJI[1,2], S. NAKAMURA[1], K. NAKAYAMA[1], A. MOCHIZUKI[1], M. KIYOMOTO[1], S. K. PARK[4], Y. C. BAE[4], M. OZEKI[2], *T. INOUE[3,1];
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Abstract: Masseter motoneurons (MMNs) have the well-developed dendrites where MMNs receive a variety of inputs. MMNs receive both glutamatergic and serotonergic inputs in the dendrites; however it is unclear how the two inputs interact with each other. In this study we examined effects of serotonin (5-HT) on the glutamatergic responses in the dendrites of the MMNs in brainstem slice preparations obtained from postnatal day (P) 2-5 neonatal rats using whole-cell recordings and laser photolysis of caged glutamate. We photostimulated 39 different spots arranged around each recorded MMN in a concave shaped array with 100 μm spacing between adjacent rows and columns. Laser photolysis of caged glutamate in the dendrites induced somatic depolarization in MMNs. Bath application of 5-HT enhanced the photostimulation-evoked depolarization and induced action potentials in the some stimulating spots. In the presence of tetrodotoxin (1 μM), the depolarizing responses were also evoked at short latencies by photostimulation. Bath application of 5-HT (0.1-100 μM) increased the amplitude of the photostimulation-evoked responses in the dendrites of the MMNs in dose-dependent manner, in addition to induction of membrane potential depolarization. The application of the 5-HT_{2A} receptor agonist TCB-2 enhanced the photostimulation-evoked responses and induced membrane depolarization. The 5-HT_{2B} selective agonist BW723C86 had no effect on MMNs. The 5-HT_{2C} selective agonist MK212 induced membrane depolarization but had no effect on the photostimulation-evoked responses. 5-HT-induced enhancement of the laser-evoked responses and the membrane depolarization were antagonized by the 5-$HT_{2A/2C}$ receptor antagonist Ketanserin. Application of the N-methyl-D-aspartate (NMDA) receptor antagonist APV blocked the 5-HT-induced enhancement, whereas application of the AMPA receptor antagonist NBQX did not affect the 5-HT-induced enhancement. Immunoelectron microscopy revealed that both NR1 subunit of NMDA receptors and 5-HT_{2A} receptors were located in the dendrites. 5-HT also enhanced the responses evoked by focal activation of glutamate receptors by two-photon uncaging of caged glutamate. These results suggest that activation of 5-HT_{2A} receptors enhanced the NMDA receptor-mediated glutamate responses in the dendrites of the neonate MMNs. 5-HT may enhance the glutamatergic motor command onto MMN during suckling.

Disclosures: **M. Dantsuji:** None. **S. Nakamura:** None. **K. Nakayama:** None. **A. Mochizuki:** None. **M. Kiyomoto:** None. **S.K. Park:** None. **Y.C. Bae:** None. **M. Ozeki:** None. **T. Inoue:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

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Program#/Poster#: 537.24/EEE13

Topic: E.10. Motor Neurons and Muscle

Support: General Stim

Title: Development and testing of nustim system for urinary stress incontinence treatment

Authors: *X. HUANG[1], L. LIAO[2], G. E. LOEB[1];
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Abstract: Pelvic floor muscle training (PFMT, also known at Kegel exercises) has been well documented to reduce urine leakage in urinary stress incontinence patients, but few patients do it properly or sufficiently. Electrical stimulation to contract weak pelvic floor muscles and external urethral sphincter (EUS) should be able to provide the same benefit as voluntary exercise. We have developed a novel, single channel, inductively powered and controlled microstimulator (NuStim®) that can be implanted percutaneously into a muscle and close to its motor axons via a minimally invasive implantation procedure. The NuStim microstimulators can be externally controlled to produce a wide range of charge-controlled stimulus intensities that can be programmed to assure complete recruitment of the motor units without voluntary effort. Power and commands are transmitted to the implant by wireless inductive coupling from a transmitter in a seat cushion. Both the clinician and the patient use an Android app to communicate wirelessly with the cushion via Bluetooth Low Energy (BLE) protocol. The complete system was validated in a chronic animal study demonstrating that the NuStim can be successfully implanted into an effective, low threshold location and the implant can be operated chronically to produce strong, stable and tolerable contraction of histologically normal skeletal muscle for up to three months. A clinical study will begin shortly to determine identify electrical stimulation patterns that are both effective and comfortable.

Disclosures: **X. Huang:** Other; General Stim. **L. Liao:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); General Stim INC. F. Consulting Fees (e.g., advisory boards); General Stim INC. **G.E. Loeb:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); General Stim INC. F. Consulting Fees (e.g., advisory boards); General Stim INC.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 537.25/EEE14

Topic: E.10. Motor Neurons and Muscle

Support: NHMRC Program Grant 1055084

Title: Motoneurone excitability of the human quadriceps muscles during a submaximal fatiguing contraction

Authors: *H. T. FINN[1], D. M. ROUFFET[3], D. S. KENNEDY[4], S. GREEN[5], J. L. TAYLOR[2]; [1]Physiol., Neurosci. Res. Australia/ UNSW, Randwick, Australia; [2]Neurosci. Res. Australia/ UNSW, Sydney, Australia; [3]Victoria Univ., Melbourne, Australia; [4]Univ. of Sydney, Sydney, Australia; [5]Western Sydney Univ., Sydney, Australia

Abstract: Introduction: The effect of fatiguing exercise on excitability of the motoneurones (MN) innervating the human quadriceps muscles is unclear. To date, assessment during ongoing voluntary activity suggests that there is little change in MN excitability (Sidhu et al., 2012). For the upper arm, MN excitability is reduced during fatiguing contractions, but this is more pronounced when tested in the absence of voluntary drive during cortical silent periods produced by transcranial magnetic stimulation (TMS) (McNeil et al., 2011). Hence, we assessed the MNs of the quadriceps muscle during cortical silent periods to determine whether their excitability decreased during a fatiguing submaximal contraction. Methods: Ten (8 male) participants attended on one (n=5) or two (n=5) days to perform brief (~5s) isometric knee extension contractions before and after a 10-min sustained contraction. Surface electromyogram (EMG) was recorded from vastus medialis (VM), vastus lateralis (VL) and rectus femoris (RF) muscles, and all contractions were performed at a target level of VM EMG activity (25% maximum). Stimuli delivered during contractions evoked responses in each muscle. Femoral nerve stimuli evoked maximal M-waves (Mmax). Electrical stimuli over the thoracic spine activated descending tracts and produced thoracic motor evoked potentials (TMEPs). TMEPs were evoked both during ongoing voluntary drive and during silent periods following TMS over the motor cortex. TMS intensity was set to cause a 200 ms period of EMG silence. Thoracic stimuli were delivered 100 ms after TMS to produce TMEPs in the absence of descending drive (TMS-TMEP). The size of the TMS-TMEP in VM was set to be large (~50% Mmax; strong stimuli; n=6) or small (~15% Mmax; weak stimuli; n=9) on the two days. Results: During the 10-min contraction the force output reduced by 53 ± 1% (mean ± SEM; $p < 0.001$). The area of TMEPs evoked by strong or weak stimulation during ongoing voluntary drive did not change in VM ($p > 0.441$) or VL ($p > 0.101$) muscles. In the RF muscle TMEP area was unchanged ($p = 0.15$) for strong stimulation, but decreased by 29 ± 4% ($p = 0.008$) for weak stimulation. The average

TMS-TMEP area over the last 5 min compared to pre-fatigue values, was reduced by 41 ± 8% ($p = 0.031$) and 63 ± 7% ($p = 0.001$) in VM, 40 ± 9% ($p = 0.046$) and 62 ± 6% ($p = 0.001$) in VL, and 37 ± 4% ($p = 0.012$) and 47 ± 6% ($p = 0.019$) in RF after strong and weak stimulation, respectively. Conclusion: TMEPs evoked in quadriceps during TMS-induced silent periods become smaller during a sustained submaximal contraction. This strongly suggests that the excitability of the motoneurones innervating the human knee extensor muscles is reduced during fatiguing exercise.

Disclosures: H.T. Finn: None. **D.M. Rouffet:** None. **D.S. Kennedy:** None. **S. Green:** None. **J.L. Taylor:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

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Topic: E.10. Motor Neurons and Muscle

Support: NIH NINDS NS089313

Flex Pharma, Inc

Title: Potential role of spinal interneurons in genesis of muscle cramps in an animal model

Authors: *M. D. JOHNSON[1], C. K. THOMPSON[2], C. J. HECKMAN[1];
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Abstract: Muscle cramps consist of repetitive involuntary firing of motor units resulting in prolonged muscle activation and can be considerably painful. Evidence from human studies suggests muscle cramps have a significant central nervous system component, but there remains substantial debate about mechanisms underlying cramps. Though it is widely assumed that animals such as horses, cats and dogs experience cramps, there is currently no effective animal research model of muscle cramping. We have developed the first animal model of cramps, using direct electrical stimulation of muscles in the decerebrate cat preparation. Using brief stimulation protocols similar to those that evoke cramps in humans, we generated strong cramp-like behaviors, with post-stimulation forces greater than 50% of the maximum that last many tens of seconds. Multi motor unit (MU) recordings, using an array electrode applied directly to the muscle, show progressive MU recruitment corresponding to cramp force development with small increases in individual unit firing. Three lines of evidence support a fundamental role for

prolonged firing of spinal interneurons in the genesis of cramps. First, motor unit coherence observed during evoked cramps is consistent with the notion that common synaptic drive to spinal motoneurons underlies muscle cramps. Secondly, reciprocal inhibition from three sources (electrical stimulation of antagonist muscle nerve, tonic stretch of antagonist muscle and dynamic stretch of antagonist muscle) is only effective in transiently inhibiting muscle cramp force, which rapidly recovers as soon as the inhibition ceases. Such transient inhibition of the cramp suggests sustained synaptic drive from segmental sources contributes to cramp activity. Finally, intracellular recordings from spinal interneurons and motoneurons suggest that the former are facilitated and the latter inhibited during direct muscle stimulus protocols used to induce cramps. Taken together, these data suggest spinal pre- motor interneurons play a significant role in the generation and maintenance of muscle cramps.

Disclosures: **M.D. Johnson:** None. **C.K. Thompson:** None. **C.J. Heckman:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 537.27/FFF2

Topic: E.10. Motor Neurons and Muscle

Support: NIH-NIAMS R01AR050520

NIH-NIAMS R01AR052345

Title: A closed-loop neuromuscular simulation suffices to explain generation and modulation of force variability in healthy adults

Authors: ***A. NAGAMORI**[1], C. M. LAINE[1], F. J. VALERO-CUEVAS[1,2];
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Abstract: Coordinated patterns of motor unit discharges reflect characteristics of the neural signals which control muscle force. Specifically, synaptic input common to concurrently active motor units is the major determinant of human force control and is associated with oscillations in force (< ~15 Hz). This neural drive can be characterized by (i) concurrent low-frequency (<5Hz) firing modulation among active motor units, often called ‘common drive’ and (ii) physiological tremor, quantified by synchronization of motor unit discharges in the 5 - 15Hz band. The neurophysiological origin of ‘common drive’ is unknown, and the factors which influence physiological tremor are actively debated. Here, we investigated the generation, modulation, and interactions between both frequency bands of neural drive using a physiologically-grounded

closed-loop simulation. Specifically, the simulation included a Hill-type muscle-tendon model, muscle spindle, Golgi tendon organ (GTO), and a feedback controller which enabled target-guided force tracking. The simulated neural drive within this model was first recorded and then fed into a simulated spiking motor neuron pool. We found that even without neural feedback, a portion of 'common drive' can emerge naturally from the intrinsic properties of motor units in reaction to a constant input, and can be amplified in the presence of noise and the activation of persistent inward currents. We further demonstrate how the interaction between mechanical properties of the muscle-tendon complex and afferent feedback serves to shape and amplify 'common drive' as well as to generate physiological tremor. Our results suggest that both the amplitude and peak-frequency of physiological tremor depend upon the gains of afferent feedback pathways (spindle and GTO feedback), and our simulation demonstrates a known, but previously unexplained, interaction between proprioceptive feedback and 'common drive'. To best of our knowledge, this study is the first to comprehensively simulate the generation and modulation of involuntary force fluctuations during the attempted generation of a constant force. The results from this study may speak to mechanisms responsible for increases in force variability during fatigue and with age as well as mechanisms of pathological conditions such as essential tremor.

Disclosures: **A. Nagamori:** None. **C.M. Laine:** None. **F.J. Valero-Cuevas:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 537.28/FFF3

Topic: E.10. Motor Neurons and Muscle

Title: Genetic identities of sub-populations of developing and mature proprioceptive sensory neurons

Authors: ***A. SHARMA**[1], H. WU[1], C. BELLARDITA[2], Y. XUAN[1], K. MELETIS[1], O. KIEHN[2], F. LALLEMEND[1];
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Abstract: Sensory feedback from skeletal muscles is essential for fine, coordinated motor control. The gatekeepers of this feedback are the proprioceptive sensory neurons (PSNs) whose cell bodies are in the dorsal root ganglia (DRG), and which are classified into three subtypes (Ia, Ib, and II) depending on their anatomy and physiology. Currently only electrophysiology allows precise discrimination between the different subtypes of PSNs, critically hindering investigations

into these important cells and the networks of which they are a part. We have been trying to elucidate genetic markers for the three different subtypes of PSNs using a powerful combination of mouse genetics, rabies virus mediated retrograde tracing, ex-vivo anatomical tracing, single cell transcriptomics, and bioinformatics. Analysis of single PSN transcriptomes has yielded several interesting populations that can be defined by their unique gene expression. Here we present our work to date in classifying these populations using in situ hybridisation with canonical and novel markers. We have tried to put these populations into the context of the sensorimotor circuit by co-labelling cells with anatomical tracing from spinal cord and different muscle groups.

Disclosures: **A. Sharma:** None. **H. Wu:** None. **C. Bellardita:** None. **Y. Xuan:** None. **K. Meletis:** None. **O. Kiehn:** None. **F. Lallemend:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

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Topic: E.10. Motor Neurons and Muscle

Support: CIHR

NSERC

Killam Trust

ALS Canada

Title: Investigating the role terminal Schwann Cells play in muscle type specific recovery following partial denervation in G93A SOD1 mice

Authors: J. M. HARRISON, *V. F. RAFUSE;
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron (MN) disease, with a prevalence of 3.9 cases in 100,000. ALS is characterized, in part, by the progressive death of MNs innervating skeletal muscles fibers. As MNs die, muscles become increasingly weaker due to progressive muscle fiber denervation. This ultimately leads to death due to respiratory failure. If muscles are partially denervated in healthy individuals due to injury, uninjured MNs sprout axons to reinnervate neighboring denervated muscle fibers. This process, known as collateral reinnervation, can restore muscle force to pre-injury values and is mediated,

in part, by terminal Schwann cells (TSCs). Collateral reinnervation is compromised in ALS, which contributes to the rate of progressive weakness. Interestingly, paralysis of fast contracting muscles occurs significantly earlier in ALS compared to slow muscles. To examine whether this is due to impaired collateral reinnervation, we partially denervated soleus (slow) and plantaris (fast) muscles in $SOD1^{G93A}$ (ALS) mice and wild type (WT) littermates by ligating and cutting the 5^{th} lumbar spinal nerve (L5) at P30 to examine the effects of partial denervation (PD) pre disease-onset. Mice were allowed to recover for thirty days to determine innervation recovery, or for three days to examine the TSC response to denervation. Our results to date show that the proportion of innervated muscle fibers in partially denervated plantaris muscles was less than similarly denervated soleus muscles in ALS mice, but not WT mice following thirty days of recovery. Furthermore, we found that a significant proportion of denervated muscle fibers in the ALS PD plantaris muscle lacked TSCs. Additionally, early results suggest that numbers of TSCs at denervated endplates decrease over time during this "pre-symptomatic" stage of disease. Interestingly, at partially innervated synapses in ALS mice, TSCs were present but often only in the region of the end plate with the axon present. Also of note, denervation in the uninjured leg was seen by P60 in the ALS mice, supporting previous findings that pathologies occur in this model before obvious motor deficits. We are currently exploring potential genomic and proteomic differences between TSCs associated with fast and slow muscles as well as between ALS and WT mice. Together, these results suggest that the sprouting capacity of fast MNs in ALS is compromised even before disease onset and that this pathology may be related to abnormalities in TSCs.

Disclosures: J.M. Harrison: None. **V.F. Rafuse:** None.

Poster

537. Motor Neurons: Activity, Sensory, and Central Control

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 537.30/FFF5

Topic: E.10. Motor Neurons and Muscle

Title: Navigated TMS to assess central motor fatigue in a fingertapping task

Authors: M. ROENNEFARTH, M. WITT, R. FLEISCHMANN, L. HABERBOSCH, A. JOOß, *S. SCHMIDT, S. A. BRANDT;
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Abstract: Motor fatigue is defined as a decline in force output. This phenomenon can occur at any stage within the motor pathway between cortex and muscle. It can be due to peripheral and central mechanisms, i.e. on a cortical, spinal, peripheral neuronal, neuromuscular or muscular

level (Edwards 1980). In analogy, a reduction of motor evoked potentials (MEP) after an initial MEP-size increase is a well-established response to transcranial magnetic stimulation (TMS) after a sufficiently long and fatiguing motor task, e.g. maximum contraction (Brasil-Neto 1993). As no change of responses to peripheral stimulation is seen this effect is interpreted as a correlate for central motor fatigue.
The Fingertapping-task (FT) represents a widely used tool to evaluate more complex aspects of motor function, higher order motor control and importantly deterioration of motor function in clinical neuroscience (Shimoyama 1990). A decline in performance corresponds to a reduction in tapping rate. Yet, parameters like session length and time of MEP assessement for the evaluation of central aspects of FT induced motor fatigue remain unclear and results have been inconclusive so far (Kluger 2012, Arias et al. 2012).
The aim of this study was to evaluate central motor fatigue as induced by FT with navigated TMS and relate electrophysiological parameters to task performance change. We aimed to identify the task length necessary to induce changes in cortico-spinal excitability after FT. Therefore 9 subjects in 5 consecutive tapping sessions with different length (15 sec, 30 sec, 60 sec and maximum possible length) in random succession order were studied. MEPs in response to navigated TMS over dominant M1 (FDI-hotspot) were recorded before, directly after (2 min continuously) and 3 minutes after each tapping session. Subjective fatigue, Intertapinterval and tapping force were recorded as well.
The task was perceived as more fatiguing when lasting longer. Tapping rate but not tapping force was significantly lower in the longer tapping sessions and decreased over time in each session. Additionally a carry over effect over session succession with an increase in tapping force was seen probably reflecting intrasession fatigue and central compensatory mechanisms ($F=9.73$, $p<0.01$). Change of tapping force was present mainly in the first up to 30sec of the respective session. A significant reduction in MEP-size as compared to baseline was present only after 60 sec and max Tapping ($p<0.05$). Thus the usual 30 seconds lasting FT seems to be too short to induce central motor fatigue that can be detected with TMS. Alternatively Tapping force could be an appropriate measure to detect subtle changes over time in FT.

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Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 538.01/FFF6

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH R01 MH103322

NIH F31 MH095253

Title: Systemic inhibition of oxytocin receptors blocks stress induced social anxiety in female California mice

Authors: *N. DUQUE-WILCKENS[1,1], M. Q. STEINMAN, 95618[2,3], P. B. TAN[3], R. HAO[3], B. C. TRAINOR[3];
[1]Animal Behavior Grad. Group, [2]Molecular, Cellular, and Integrative Physiol. Grad. group, [3]Psychology, Univ. of California, Davis, Davis, CA

Abstract: Oxytocin (OT) is often considered as pro-social and anxiolytic, but recent evidence suggests that the effects of OT are context-specific. It has been proposed that OT increases the salience of social cues, which can explain why OT can either enhance or inhibit social behaviors. We recently discovered that social defeat stress induces hyperactivity in OT neurons in the bed nucleus of the stria terminalis (BNST) and paraventricular nucleus, and that this effect is primarily observed in females but not males. These studies used the monogamous California mouse, which is one of the few species in which social defeat stress can be studied in both males and females. We found that intranasal infusions of OT (INOT) reduced social interaction behavior in females that are naïve to defeat, which mirrors the effects of social defeat stress on this behavior in females. Here we used Egr-1 expression to assess the potential neuroanatomical substrates underlying the sex specific response to INOT. We found than in females but not males naïve to defeat, INOT increases Egr-1 binding in nucleus accumbens core (NAcc) and anterior BNST, two areas previously associated with stress-induced social withdrawal. Next we used a pharmacological approach to determine whether increased activation of OT receptor (OTR) in stressed females contributes to stress-induced decreases in social interaction behavior. Control and stressed females were randomly assigned to receive an IP injection of saline or OTR antagonist (L-368,899, Sigma) 30 min before behavior testing. Control females showed high levels of social interaction (SI), and in an odor preference test (OP) preferred an odor of an unfamiliar individual vs. the familiar odor of a cagemate. Stress not only reduced SI, but also reversed the OP such that stressed females preferred the odor of a familiar cagemate over an unfamiliar odor. Interestingly, OTR antagonist treatment rapidly blocked the effects of stress on both SI and OP. This suggests that stress-induced increases in OTR activation, possibly in NAcc and anterior BNST, may drive females away from unfamiliar and towards familiar (and possibly safer) social contexts. These results suggest that the use of OTR antagonists could have unappreciated benefits as an anxiolytic agent in social contexts.

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Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 538.02/FFF7

Topic: F.02. Behavioral Neuroendocrinology

Title: *In vitro* and *In vivo* pharmacological profiles of non peptidic selective oxytocin receptor ligands

Authors: *J. BECKER[1], J. GANDIA[1], L. PELLISSIER[1], I. KARPENKO[2], D. BONNET[2], M. HIBERT[2], J. LE MERRER[1];
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Abstract: The neuropeptide oxytocin plays a crucial role in controlling many aspects of social behavior, such as social cognition and empathy, sexual, parental and affiliative behaviors or aggressiveness. In this context, deficient oxytocin activity has been linked to several neuropsychiatric disorders, including schizophrenia, drug addiction and autism spectrum disorders (ASD). Oxytocin binds its G protein coupled receptor (OTR), as well as arginine vasopressin receptors (V1A, V1B and V2). To disentangle the respective contributions of OTR and vasopressin receptors in mediating the oxytocin effects on social behaviour, there is a crucial need for developing brain penetrant, stable and selective agonists and antagonists of OTR. We present here the *in vitro* pharmacological profiles of newly synthesized compounds targeting selectively OTR and their *in vivo* activity in a mouse model of ASD.

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Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

Location: Halls B-H

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Program#/Poster#: 538.03/FFF8

Topic: F.02. Behavioral Neuroendocrinology

Support: University of Melbourne Early Career Researcher Grant # 601692

Title: Cerebrospinal fluid oxytocin according to the social context and a body pressure jacket

Authors: *J.-L. RAULT;
Fac. of Vet. and Agr. Sci., Univ. of Melbourne, Parkville, Australia

Abstract: Oxytocin (OT) underlies numerous socio-behavioral processes as a neuropeptide. Nevertheless, the sensitivity of the endogenous oxytocinergic system to changes in the social environment remains poorly understood. This is mainly due to the technical difficulty to sample central OT and the methodological difficulty to validate reliable proxy measures of central OT. This study investigated the effects of varying social contexts and a body pressure jacket on OT concentration in the cerebrospinal fluid (CSF). The pig (*Sus scrofa*) was used as a model, with repeated CSF sampling through a spinal catheter using a within-subject design. The pigs were subjected to two tests two days apart. The first test was a three phases Union-Separation-Reunion paradigm to assess the effects of social presence and social separation and isolation on CSF OT concentration, with each phase lasting 30 min and two companion pigs present for the Union and Reunion phases. The second test consisted of fitting a jacket on the pigs (ThunderShirt®) that provided gentle body pressure and is believed to mimic the relaxing response seen in pigs, humans and other animal species following gentle body squeeze. The hypothesis was that the jacket may stimulate central OT release, as physical contact can trigger OT release and OT can have anxiolytic effects. The present findings showed that the jacket resulted in inconsistent individual differences in CSF OT, with one individual showing a 3 fold increase in CSF OT after 10 min of wearing the jacket but also an hour after the jacket was removed, while another individual remained at baseline level. The jacket effect requires research with a larger sample size. The Union-Separation-Reunion social testing paradigm did not result in consistent changes in CSF OT, but CSF OT changes were positively correlated with explorative behaviors (root, manipulate) in a novel environment in presence of conspecifics. Accumulating evidence supports that OT actions are context-specific, possibly explaining the discrepancy in the literature on OT and its (sometimes contradictorily) relationship with positive or negative social behaviors. In the present experiment, CSF OT inconsistent concentration changes could be partly explained by simultaneously considering behavior in response to the context, as CSF OT concentration related to exploratory drive in the social paradigm. The influence of the context ought to be considered in OT research given the multitude of factors that may affect the oxytocinergic system or potentiate its effects. This should help identify effective and repeatable clinical interventions or situations conducive to endogenous central OT release.

Disclosures: J. Rault: None.

Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 538.04/FFF9

Topic: F.02. Behavioral Neuroendocrinology

Title: An eye tracking system revealed the face scanning pattern and the effect of oxytocin for enhancing eye contact in common marmosets

Authors: M. KOTANI[1], K. SHIMONO[2], T. NAKAKO[1], Y. IWAMURA[1], H. IMAI[1], A. KIYOSHI[1], K. MATSUMOTO[1], A. MATSUMOTO[1], M. IKEJIRI[1], T. NAKAYAMA[1], Y. OGI[1], N. KONOIKE[4], K. NAKAMURA[4], *T. ISHIYAMA[3], K. IKEDA[1];
[1]Higher brain function Res., [2]Mol. Pathophysiology Res., [3]Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan; [4]Dept. of Neuroscience, Primate Res. Inst., Kyoto Univ., Aichi, Japan

Abstract: Introduction Eye contact is a useful biomarker of social communication and known to be deficient in patients with some psychiatric conditions. An eye tracking system has been used to investigate the face scanning pattern in patients with autism spectrum disorders (ASDs) and found that oxytocin, a neuropeptide that regulates social behavior and cognition, increased the gaze to the eye region of human face (presumed as eye contact). Interestingly, eye tracking has been used to directly compare the face scanning in some non-human primates with that in human. Thus, eye tracking is expected to be a translational technique between human and non-human primates to evaluate the effect of psychiatric drug candidates. Here we have established the method of eye tracking in common marmosets, unique New World primates that resembles human's behavioral characteristics such as eye contact as a means of communication, in order to firstly characterize their face scanning pattern and evaluate the oxytocin's effect for eye contact. Materials and methods Head posts were surgically implanted in five male adult marmosets and used to stabilize their heads through pretest and test. Oxytocin of the common marmoset was synthesized and intranasal administration of vehicle or 100IU oxytocin was made in a cross-over manner, immediately and thirty minutes before pretest and test, respectively. For pretest and test, common marmosets were free to view close-up pictures of conspecific face for 5 s on the computer screen, and their eye positions were continuously recorded with the infrared eye tracking camera (ETL-400, ISCAN Inc.). Viewing time for the eyes, the mouth, and the other face parts (the face) in a picture were measured, and the ratio of viewing time for eye or mouth to face was calculated. T-test was done for any statistical comparisons. All experimental procedures involving animals use were reviewed and approved by the Institutional Animal Care and Use Committee of Sumitomo Dainippon Pharma, Co., Ltd. Results The viewing time in non-treated marmosets was apparently longer ($P = 0.003$) for the eyes (2.24 s, 59.58% as eye/face ratio) than for the mouth (0.32 s, 9.29% as mouth/face ratio). In oxytocin treatment study, only the change of eye/face ratio from pretest to test was significantly greater ($P = 0.011$) in oxytocin group than

in vehicle group. Conclusion We could successfully measure the unique eye contact behavior in common marmosets with the newly-established method of eye tracking and demonstrate that oxytocin enhanced the eye contact. This system will be useful for further testing of therapeutic compounds for psychiatric conditions, especially including ASDs.

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Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 538.05/FFF10

Topic: F.02. Behavioral Neuroendocrinology

Support: Western University of Health Sciences

Title: Expression of neuropeptide arginine vasopressin and its receptors 1a and 1b in the canine amygdala

Authors: ***G. KAUR**[1], M. SAILOR[1], H. MIRRASHED[1], F. SHAHRIAR[2], W. KHAMAS[1]; [1]Western Univ. of Hlth. Sci., Pomona, CA; [2]Animal Diagnos. Lab., Tustin, CA

Abstract: Neuropeptide arginine vasopressin (AVP) is known to modulate social behavior in several species. The AVP receptors 1a (V1a) and 1b (V1b) are broadly distributed in the limbic system of rats, mice and hamsters, with several studies documenting their involvement in behavior regulation. AVP has been shown to modulate aggression by acting through V1a and/or V1b receptors in hamsters, rats and mice. However, the role of AVP and its receptors in canine aggression is unknown, due to lack of studies and species differences. Here, as the first step, we investigated the presence of AVP, V1a, and V1b in the canine amygdala by using immunohistochemistry and Western blot analysis on post-mortem canine brain tissue. Immunohistochemistry applied to freely floating canine brain tissue sections successfully identified the presence of neurons immunoreactive for AVP, V1a, and V1b in the amygdala of both sexes. Following validation of protein extraction from formalin fixed canine tissue, the expression of AVP and receptors was confirmed successfully by Western blotting. The identification of AVP, V1a, and V1b immunoreactive neurons in the canine amygdala suggests a potential role in regulating social behaviors including aggression in domestic dogs. This study is the first demonstration of the presence of AVP and its receptors in the canine amygdala. The data

from this and subsequent studies may lay groundwork for research to design novel therapeutic interventions to regulate canine behavioral problems.

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Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

Location: Halls B-H

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Program#/Poster#: 538.06/FFF11

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R15MH100585

Title: Play and social approach in juvenile F344 and Lewis rats: Effects of oxytocin in the central amygdala

Authors: S. R. ECK, L. A. MCDOWELL, *S. M. SIVIY;
Dept Psychol, Gettysburg Col., Gettysburg, PA

Abstract: Previous work from our laboratory has shown that the Fischer 344 (F344) rat is consistently less playful than other inbred and outbred strains and we have been recently investigating the extent to which oxytocin (OT) may be contributing to the differential levels of playfulness in this strain. Accordingly, we have begun to assess the effects of intracranial infusions of OT into limbic structures thought to be involved in play and that have abundant OT receptors. In an initial study (SFN 2015) we reported that oxytocin (0.5 µg/0.5 µl) infused into the central amygdala (CeA) had no effect on play in either F344 or Lewis rats but resulted in a robust increase in self-grooming that was especially prominent in the Lewis strain. Since OT-induced hyper-grooming may have obscured any potential effect of OT on play we looked at the effects of lower doses of OT when infused into the CeA of male and female juvenile F344 and Lewis rats. Rats were isolated for 4 hours prior to testing with an untreated Sprague-Dawley partner for 10 minutes. Two doses of OT (10, 50 ng) plus a vehicle control were assessed over 3 test days and infused (0.5 µl over 1 minute) 5 minutes before testing. Each inbred rat received each dose in a counter-balanced order. Play was quantified primarily by the frequency of pounces and pins directed towards the SD partner. Amount of time spent in active social investigation (sniffing, grooming) as well as time spent in self-grooming were also measured. As expected, F344 rats exhibited fewer pounces and pins than Lewis rats. F344 rats were also less likely to engage in self-grooming than Lewis rats. OT infusions had no effect on either measure of play, nor did it affect either social investigation or self-grooming. In order to confirm the

reliability of the hyper-grooming effect all of the rats were subsequently infused with 500 ng (0.5 µg)/0.5 µl as in our previous study. As expected, this dose of OT yielded a substantial amount of self-grooming that was especially prominent in Lewis rats. These data, combined with our earlier findings, suggest that OT in the CeA may not have a significant modulatory influence on overall levels of playfulness in the juvenile rat, nor can differential sensitivity to OT within this area account for the strain differences observed in play behavior. However, the data do point towards a robust strain difference in the extent to which OT can induce self-grooming when infused into the CeA. In order to further examine any prosocial effects that may be associated with OT in juvenile rats we have also begun to assess strain differences in a more selective test of social approach and will be assessing the effects of OT in this model as well.

Disclosures: **S.R. Eck:** None. **L.A. McDowell:** None. **S.M. Siviy:** None.

Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIMH Intramural Research Program ZIA-MH-002498-24

Title: Nasal oxytocin in a genetic knockout: Pharmacokinetics and behavior

Authors: *A. S. SMITH[1], A. C. KORGAN[2], J. FASTMAN[3], M. C. GRANOVETTER[3], J. SONG[3], W. S. YOUNG[3];
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Abstract: The hormone oxytocin (Oxt) has been shown to promote social behavior in numerous mammalian species. This has led to its use as a therapeutic to target human conditions characterized by deficits in social functioning, especially autism spectrum disorder and schizophrenia. One significant conundrum has been how to deliver Oxt to the brain without resorting to invasive administration procedures, as only 1% of an injected, peripheral dose of Oxt crosses the blood-brain barrier. Intranasal administration of Oxt has become the primary non-invasive delivery route, from basic research to experimental clinical trials. Despite the growing number of human studies using the nasal route of administration, there is no direct evidence for central uptake of Oxt from this administrative route and limited knowledge regarding the effect of chronic application on behavior and the endogenous Oxt system. Such information is vital to

inform the efficacy, tolerability, and safety of intranasal-administered Oxt in humans. Here, we provide a detailed pharmacokinetic examination of nasal-administered Oxt concurrently in the circulatory and central nervous systems of mice. Most significantly, we utilized an Oxt knockout mouse to monitor the distribution of nasally delivered Oxt in the absence of endogenous production of Oxt to validate the origin of the Oxt being monitored. Our study demonstrates for the first time that nasal administration of Oxt permeates into specific areas of the brain. Furthermore, chronic nasal Oxt administered every other day can increase social behavior, particularly olfactory-based social investigation, and boost Oxt receptor production in brain regions associated with the olfactory system. These effects depend on the dose administered. By contrast, chronic daily nasal Oxt suppresses social behavior and Oxt receptor expression in the brain. Thus, chronic nasal Oxt has impacts on behavior and the endogenous Oxt system in an intriguing dose- and schedule-specific manner. These data have interesting implications for the interpretation of various behavioral and physiological effects described in animal and human studies after nasal administration of Oxt and provide the pharmacokinetics necessary to rationally develop this drug delivery route for therapeutic purposes.

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Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

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Topic: F.02. Behavioral Neuroendocrinology

Support: Chica and Heinz Schaller Research Foundation, Human Frontiers Science Program RGP0019/2015, DFG within the Collaborative Research Center (SFB) 1134 and 1158

Title: Evoked oxytocin release in the medial prefrontal cortex facilitates social interaction in female rats

Authors: ***A. RAFTOGIANNI**[1,2], S. MELZER[3,4], M. DA SILVA GOUVEIA[1], M. ELIAVA[1], S. H. KNOBLOCH-BOLLMANN[1,5], P. H. SEEBURG[2], H. MONYER[3], V. GRINEVICH[1,6]; [1]Schaller Res. Group On Neuropeptides At DKFZ, Heidelberg, Germany; [2]Mol. Neurobio., Max Planck Inst. for Med. Res., Heidelberg, Germany; [3]Dept. of Clin. Neurobio., Heidelberg Univ. and German Cancer Res. Ctr. (DKFZ), Heidelberg, Germany; [4]Dept. of Neurobio., Howard Hughes Med. Institute, Harvard Med. Sch., Boston, MA; [5]Dept. of Mol. and Cell. Biol., Ctr. for

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Abstract: Oxytocin (OT) is the neuropeptide synthesized by neurons of the paraventricular and supraoptic nuclei of the hypothalamus. It is well established that OT within the brain acts as a neuromodulator and plays an important role in reproduction, social recognition, pair bonding, maternal behavior, anxiety, and generally is involved in a wide variety of social behaviors in diverse species. The medial prefrontal cortex (mPFC) is one of the key brain regions, which coordinates social behavior and also a potential target for OT. In the present work we focused on anatomical and functional connectivity of OT neurons within mPFC, specifically infralimbic cortex (IL), as well as on the effects of OT release within the IL on social behavior. Using latex beads and viral vectors we showed that there is a direct projection of OT neurons to the IL. In addition, by using transgenic OTR-reporter animals and immunohistochemistry, we revealed that the vast majority of cells expressing OT receptors in the IL were GABAergic neurons. Furthermore, by applying viral-mediated expression of channelrhodopsin 2 (ChR2) in OT neurons and their axons (Knobloch et al., 2012; Eliava et al., 2016), we recorded cellular responses of individual neurons in acute slices of the IL. The whole-cell recordings revealed that blue light illumination of OT axons carrying ChR2, resulted in an increase of IPSC frequencies in subpopulations of GABAergic and pyramidal neurons of the deep layers. Recently, in order to investigate the behavioral effects of endogenous OT within IL, we subjected female rats to social interaction test. Prior to the test, the rats were injected with a virus expressing ChR2 under the control of OT promoter and implanted with optic fibers above the IL. The control animals expressed Venus instead of ChR2. Preliminary results suggest that rats expressing ChR2 spent more time interacting with unknown conspecifics compared to control rats after blue light illumination of the IL in both groups. In conclusion, OT facilitates social behavior in female rats via activation of GABAergic interneurons, suggesting that the inhibition/excitation balance within local IL network is an important factor, which can modulate “the degree” of pro-social behavior. Furthermore, numerous studies demonstrated that intranasal OT application in humans can change mPFC activity, thus the dissected hypothalamic-mPFC functional circuit might help in the understanding of neuronal mechanisms underlying socio-emotional deficits in human patients afflicted with psychiatric disorders.

Refs
Knobloch et al., Neuron, 2012
Eliava et al., Neuron, 2016

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Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

Location: Halls B-H

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Topic: F.02. Behavioral Neuroendocrinology

Title: Eeg response to negative visual images differs across oxytocin receptor subtype

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Abstract: The brain rapidly evaluates incoming visual information. Emotionally charged images stimulate greater neural response compared to emotionally neutral input, with negatively valenced images creating the most intense response. Studies report that emotional behaviors differ as a function of a person's oxytocin receptor genotype for the rs53576 single nucleotide polymorphism. Measuring visually evoked EEG responses recorded at the PZ electrode placement, negative images stimulated a higher peak response in the early phase (250 -450 msec post stimulus) of the Late Positive Potential (LPP) in people who have the GG genotype compared to those with either the combined GA / AA genotype. ($t(35) = 3.63$, $p < .05$). The mid phase (450 - 650 msec post stimulus) of the LPP was not statistically different between genotypes, nor was the late phase of the LPP (650 - 900 msec post stimulus). For the mid phase of the LPP, there was a significant correlation between the participants scores on a self-report altruism scale and their peak EEG response ($r(35) = .432$, $p < .05$). Cardiac response to visual image presentation will also be measured as a function of genotype and self-report altruism score.

Disclosures: D.S. Albeck: None. **K. Braley:** None. **E. Russell:** None. **J.J. Fowler:** None. **R.J. Jirsaraie:** None. **H. Mamo:** None. **C. Phiel:** None. **M. Kisley:** None. **R. Farero:** None. **R. Farero:** None.

Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

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Topic: F.02. Behavioral Neuroendocrinology

Support: This research was supported by the intramural research program of the NIMH (ZIA-MH-002498-24).

Title: Social tuning of vasopressin 1b receptor-expressing pyramidal neurons in CA2 hippocampal subfield of mice

Authors: *A. CYMERBLIT-SABBA, M. C. GRANOVETTER, S. WILLIAMS AVRAM, A. S. SMITH, J. FASTMAN, H.-J. LEE, J. SONG, W. S. YOUNG;
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Abstract: The idea that the hippocampus provides a neural basis for cognitive mapping has served as the starting point for many studies that demonstrated its involvement in encoding the map of an individual's spatial location. Nevertheless, these highly important findings present a narrow approach to the original proposal, limiting the hippocampus to spatial organization. Although still elusive, the hippocampus involvement in social cognition is of great interest for social neuroscience. The CA2 subfield has become a focus of recent research due to its unique characteristics and distinct patterns of connectivity with other brain regions. Together with selective expression of the vasopressin 1b receptor (Avpr1b) for the 'social' neurohormone vasopressin, it plays a crucial role in social memory and behavior. By activating GCaMP6s expression in Avpr1b-*Cre* recombinase transgenic mice, we imaged the calcium transients in selective pyramidal neurons within the CA2 subfield while utilizing well-established behavioral paradigms, such as social recognition and social habituation/dishabituation. Our results indicate these cells are responsive to social stimulation, with sparser representation upon repetition. In the neural population imaged, a subset of cells initially demonstrates increased firing rate when the mouse is presented with a social stimulus followed by a significant decrease across repeated exposures. Since a global reduction in firing rate in the entire population is not observed, the effect appears to be stimulus-specific. Further decoding this neural activity in the CA2 will deepen the understanding of the social neural network.

Disclosures: **A. Cymerblit-sabba:** None. **M.C. Granovetter:** None. **S. Williams Avram:** None. **A.S. Smith:** None. **J. Fastman:** None. **H. Lee:** None. **J. Song:** None. **W.S. Young:** None.

Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

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Program#/Poster#: 538.11/FFF16

Topic: F.02. Behavioral Neuroendocrinology

Title: Temporal aspects of social recognition memory in oxytocin receptor knockout mice

Authors: *T. V. MILLER, B. DONELL, H. K. CALDWELL;
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Abstract: Oxytocin (Oxt) is a neuropeptide important for displays of social behaviors in many mammalian species and has been implicated in the neural modulation of social memory. In rodents, social recognition memory is olfactory-dependent and important for context-appropriate displays of social behaviors. Tests for social memory in mice are predicated on their preference to spend time investigating odor cues from novel conspecifics rather than familiar conspecifics. It is well established that mice with either a genetic disruption of Oxt or the Oxt receptor (Oxtr) display a form of social amnesia; though, in Oxtr knockout (Oxtr -/-) mice that social amnesia does not manifest with a ten minute retention interval in a habituation-dishabituation test, but does with a thirty minute retention interval in a two-trial social discrimination test. However, these retention intervals have yet to be manipulated within the same test. In order to determine the temporal parameters of social memory in Oxtr -/- mice, we performed a two-trial social discrimination test and varied the retention interval (10 min, 20 min, and 30 min). We found that while wild type controls have normal social recognition memory, being able to distinguish novel from familiar stimulus animals at all of these retention intervals, Oxtr -/- mice do not appear to have social recognition memory with any of the retention intervals. These data suggest that social memory in Oxtr -/- mice is flexible and depends on the difficulty of the behavioral test.

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Poster

538. Social Behavior: Effects of Oxytocin and Vasopressin

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Title: Oxytocin signaling in the nucleus accumbens modulates correlated activity across forebrain nuclei during mating in male prairie voles

Authors: *Z. V. JOHNSON[1,2], H. WALUM[2], L. KING[2], Y. XIAO[2], P. RIEFKOHL[2], L. YOUNG[2];
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Abstract: Oxytocin receptors (OXTRs) modulate vertebrate social behavior and exhibit diverse patterns of central expression both across and within species. Recent models hypothesize that neuropeptide receptors (e.g. OXTRs) modulate distributed patterns of functional coupling across conserved neural networks during social contexts, but few experiments have tested this hypothesis. OXTR in the nucleus accumbens (NAcc) plays a role in social reward in mice and pair bond formation in monogamous prairie voles. OXTR density in the NAcc has been associated with species differences and individual variation in social behaviors associated with monogamous mating systems. We hypothesized that variation in OXTR signaling in the NAcc of prairie voles would result in variation in functional coupling across an associative social olfactory learning network in the brain. Using Fos immunoreactivity as a proxy of neural activation, we applied pharmacological and genetic approaches to investigate whether variation in accumbal OTR signaling modulates functional coupling across this network in male prairie voles during sociosexual interaction. We find that site-specific blockade of OTR in the NAcc decreases coupling (i.e. correlated Fos expression) between the NAcc shell and other social olfactory, limbic, and mesolimbic reward nuclei ($p=0.02$) during sociosexual interaction in male prairie voles. Next, we use a naturally occurring polymorphism in the prairie vole OXTR gene (*Oxtr;* NT213739) that strongly predicts individual variation in NAcc OXTR binding to show that genetic *Oxtr* variation associated with NAcc OXTR density is also associated with different patterns of coupling across the network ($p<0.05$) during sociosexual interaction. Collectively, these data support previously hypothesized links between central neuropeptide systems and neural network function during social contexts, and provides a mechanism by which OTR signaling may modulate salience and reinforcing value of social stimulidurin pair bond formation.

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Poster

539. Sexual Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: Canadian Institutes for Health Research (CIHR)

Title: Conditioned partner preference in male and female rats for a somatosensory cue

Authors: *G. R. QUINTANA ZUNINO[1], S. DESBIENS[2], S. MARCEAU[2], N. KALANTARI[2], J. BOWDEN[2], J. G. PFAUS[2];
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Abstract: We have shown previously that male and female rats form conditioned preferences for sex partners bearing an odor paired previously with either the postejaculatory reward state of the male or the reward state induced by paced copulation in the female. The odor thus acts as a discrete, partner-related cue for sexual reward. The present experiment examined whether a somatosensory cue (a rodent tethering jacket) could act similarly as a discrete partner-related cue. In the first study, 60 sexually-naïve Long-Evans males and females underwent 14 optimal conditioning trials with their opposite-sex partners in unilevel pacing chambers. Paired males were trained using a 1-hole Plexiglas divider with sexually receptive females wearing the rodent tethering jacket. Explicitly-paired females were given 14 trials in sequential order in the same chamber. Half of the trials were conducted using a 4-hole Plexiglas divider always associated with males wearing the jacket, and the other half without the divider associated with males not wearing the jacket. On the final test, experimental males were placed into a large open field with two sexually receptive females, one wearing the jacket and one without the jacket. Experimental females were placed into the same open field with two Plexiglas dividers with six holes each. A male with the jacket was placed on one side, and a male without the jacket was placed on the other side, and the female could pass freely from the central area to one side or the other. Results showed that males ejaculated first more times with the females wearing the jacket relative to females not wearing the jacket, similar to the results obtained previously for females bearing odor cues. However, females had no significant preference for males with or without the jacket. In the second study, 30 males underwent seven trials with sexually receptive jacketed females and seven trials with non-sexually receptive unjacketed females. 30 females were also tested in which the unjacketed male was a long-term castrated male and thus sexually nonreceptive. The final open field test was run identically to the first experiment. Both experimental males and females displayed a significant preference for the partner with the jacket relative to the partner without the jacket. Following this test, two more conditioning trials were conducted with partners wearing the jacket. These results demonstrate that a somatosensory cue can become a

discrete, partner-related conditional stimulus for sexual reward. However, it is easier to establish this relationship in males than females, who seem to require the jacket to differentiate sexually receptive versus nonreceptive partners explicitly.

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Poster

539. Sexual Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: RO1 MH50388

C.A.C. is a F.R.S.-FNRS Research Associate

Title: Preoptic glutamate and estradiol release during male sexual behavior

Authors: C. DE BOURNONVILLE[1], M.-P. DE BOURNONVILLE[2], N. AOURZ[3], A. VAN EECKHAUT[3], I. SMOLDERS[3], G. F. BALL[4], J. BALTHAZART[2], *C. A. CORNIL[2]; [1]Univ. of Massachusetts, Amherst, MA; [2]GIGA Neurosciences, Univ. of Liege, Liege, Belgium; [3]Ctr. for Neurosciences (C4N), Vrije Univ. Brussel, Brussels, Belgium; [4]Dept. of Psychology, Univ. of Maryland, College Park, MD

Abstract: Beside its long-term control by steroids, male sexual behavior is also modulated by neuroestrogens in a dynamic way (within minutes) in a number of species ranging from fishes to mammals. Studies in male Japanese quail have also identified following exposure to a receptive female a rapid decrease in the activity of brain aromatase (AA) the enzyme responsible for the conversion of androgens into estrogens. These effects occur mainly within the medial preoptic nucleus (POM), a sexually dimorphic structure of the preoptic area that plays a key role in the activation of male sexual behavior and contains the highest AA in the brain. *In vitro* studies demonstrated that AA can be rapidly inhibited by calcium-dependent phosphorylations of the enzyme triggered by the activation of AMPA and kainate receptors. We confirmed here this rapid effect of glutamate on AA by injecting kainate in the POM of anesthetized males and measuring AA in the tissue after brain collection. AA in POM was inhibited in the kainate-injected hemisphere compared to the control hemisphere injected with vehicle. In a second experiment, we showed by *in vivo* microdialysis that glutamate is released in POM during copulation. These results thus suggest that glutamate controls dynamic changes of AA that occur

in the brain during sexual interactions. To confirm that the decrease in AA leads to an actual reduction of local estradiol concentration, we quantified via microdialysis and radioimmunoassay changes in estradiol concentration in the male POM during sexual interactions with a female. Surprisingly, a dramatic elevation of estradiol was observed during copulation. Estradiol has been shown to enhance acutely male sexual motivation, therefore the function of its increase in the POM could be to maintain motivation during the entire sexual encounter. The decrease of AA observed *ex vivo* after copulation would then reflect a compensatory mechanism to restore baseline pre-copulatory conditions. Importantly, these results highlight that although long-term changes in AA are often used as a proxy for local estradiol concentrations, these two measures can show major short-term discrepancies possibly reflecting variations in estrogen turnover.

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Poster

539. Sexual Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Title: Sexual experience influences gene expression pattern of corticotropin-releasing hormone (CRH) and vgf nerve growth factor inducible (VGF) in the medial preoptic nucleus (MPN) in male rats

Authors: *S. MAEJIMA, S. YAMAGUCHI, K. UCHIYAMA, M. MORISHITA, S. TSUKAHARA;
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Abstract: The MPN is a regulatory center of male sexual behavior and consists of male-biased sexually dimorphic structures that are found in the central part of the MPN (MPNc). It is suggested that the function of the MPNc involved in male sexual behavior is dependent on sexual experience, although the mechanisms are largely unknown. We previously found that the MPNc highly expressed c-Fos protein in male rats that displayed sexual behavior followed by ejaculation, and this increase was higher in males having first ejaculation than in males having second ejaculation. These results suggest that neural activity of the MPNc is modulated by sexual experience. In this study, we performed DNA microarray analysis of male rats displaying sexual behavior followed by ejaculation to determine the effects of sexual experience on gene

expression in the MPNc. In first ejaculation-experienced males, mRNA expression of CRH and VGF were significantly increased and these increases tended to decrease after second ejaculation. Next, we performed morphological analyses to examine whether CRH and VGF neurons in the MPNc are involved in the sexual experience-dependent regulation of sexual behavior. Brain sections obtained from male rats, which experienced ejaculation, were subjected to immunohistochemistry of CRH and *in situ* hybridization of VGF mRNA with combination with c-Fos-immunohistochemistry. Immunohistological analysis of CRH revealed that the MPNc contained CRH-immunoreactive cell bodies and fibers, and CRH-immunoreactive signals were decreased by ejaculation with or without sexual experience. These results suggest that CRH in the MPNc is released by ejaculation stimuli independently of sexual experience. On the other hand, histological analyses of VFG mRNA and c-Fos protein showed that the increased expression of VGF mRNA and c-Fos protein in the MPNc with ejaculation was higher in males when they had first ejaculation compared with second ejaculation. However, most of VGF neurons did not express c-Fos protein. These results suggest that the VGF-expressing neurons in the MPNc are involved in male sexual behavior in a sexual experience-dependent manner, although there may be other neuronal populations, the neural activity of which is changed by sexual experience.

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Poster

539. Sexual Behavior

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Topic: F.02. Behavioral Neuroendocrinology

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Title: Testosterone increases soma size of neurons in the posterodorsal medial amygdala in a cell-autonomous manner

Authors: ***N. J. HOBBS**, C. L. JORDAN, S. M. BREEDLOVE;
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Abstract: The posterodorsal medial amygdala (MePD) is a sexually dimorphic brain region in adult rodents. Previous work in our lab found that circulating androgens such as testosterone (T) maintain sex differences in the volume of the MePD as well as neuronal soma size, with males having a larger MePD with larger neurons. These androgen responses are mediated by the

androgen receptor (AR) since Tfm rats and mice with dysfunctional AR are demasculinized in these measures. However, it is unclear if this increase in cell size in wildtype animals is an effect of T acting directly on the cell via the AR or if it is an indirect effect of T acting on neurons in other brain region(s) connected to the MePD, such as the preoptic area (POA). Therefore we conducted a mosaic analysis of AR+ and AR- cells in the MePD of wildtype mice to determine if there is a cell-autonomous effect of T on cell size mediated by AR. Male mice were castrated at 60 days of age and given either a Silastic capsule containing free T or a blank capsule. Thirty days later, brains were collected and sectioned. Tissue underwent AR immunohistochemistry and was counterstained with thionin to stain for Nissl substance. We found that MePD neurons were larger in castrates given testosterone than those given blanks. In blank-treated animals there was no difference in the soma sizes of AR+ versus AR- neurons. However, analysis of T-treated animals revealed that only AR+ cells increased in size in response to T. AR- cells in T-treated mice were similar in size to both AR+ and AR- cells in blank-treated control mice. These results indicate that androgens acts directly on MePD neurons to increase their size in a cell-autonomous manner since only AR+ cells responded to the hormone. A similar effect was seen in the motoneurons of the spinal nucleus of the bulbocavernosus of rats (Watson et al., 2001). These results indicate androgen acts within the MePD to alter its anatomy and so may also affect the region's function in analyzing pheromonal and olfactory stimuli.

Disclosures: **N.J. Hobbs:** None. **C.L. Jordan:** None. **S.M. Breedlove:** None.

Poster

539. Sexual Behavior

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UNAM- DGAPA-PAPIIT IN203615,IN210215

Instituto Nacional de Perinatología 212250-3230- 21216-05- 15

Title: Characterization of the effect of experience on sexual behavior in the prairie vole

Authors: *T. E. AGUILAR[1], N. F. DIAZ[2], M. N. ULLOA[3], L. J. YOUNG[4], R. G. PAREDES[3], W. PORTILLO[3];
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Abstract: The prairie vole (*Michrotus ochrogaster),* is a monogamous mammal. It has been shown that cohabitation with mating for 6h induces a pair bond that in most cases is permanent. However, a detailed characterization of the components of sexual behavior in relation to experience in prairie voles has not been reported. The current study further quantifies components of sexual behavior in prairie voles and evaluates whether sexual experience modifies the expression of sexual behavior. To this end, we compared female and male sexual behavior during three sessions of one or six hours, respectively, with an interval of 24 hours between each session. For females, lordosis response was measured and reported as the lordosis coefficient (LQ). Male sexual behavior was registered including the number and latency to mount (NM, ML), intromit (NI,IL) and ejaculate (NE,EL), in addition the interintromission (III) and postejaculatory interval (PEI) were calculated. Thirty two voles (sixteen males and sixteen females), with no sexual experience were used in this study. All females were ovariectomized and supplemented with estradiol during nine days to induce and maintain sexual receptivity during the three behavioral tests; males remained gonadally intact. There were no significant differences between the sexual behavior parameters in either males or females during the three sessions in which they mated for 1 h with the same partner. On the other hand, in the 6 h tests the intromission latency was significantly reduced in the second and third test in comparison to the first test. These results indicate that contrary to what is observed in rats and mice, voles do not show an improvement in the mating pattern associated with experience. This research was supported by grants CONACYT 252756, 167101; Fronteras 374; UNAM-DGAPA-PAPIIT IN203615, IN210215; Instituto Nacional de Perinatología 212250-3230-21216-05-15. We thank Francisco Camacho and Deisy Gasca for their technical assistance.

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Poster

539. Sexual Behavior

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PRODEP Neuroendocrinology BUAP-CA-288

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Title: Effect of maternal care on the display of copulatory behavior in the subline that differ in spontaneous yawning

Authors: *A. DORANTES, C. CORTES, A. UGARTE, J. R. EGUIBAR;
Inst. de Fisiología, Benemérita Univ. Autónoma De Puebla, Puebla, Mexico

Abstract: Sexually experienced male rats usually ejaculates after 10 to 15 intromissions in about 7 to 15 min. Males with a long delay to ejaculate or fail to reach it in tests lasting 30 min are sluggish (S), but if they did not copulate (without mounts) are non-copulating (NC) males. High-yawning (HY) rats had a higher percentage of S and NC than Sprague-Dawley (SD) rats. Additionally, HY and LY (low-yawning) dams have a different maternal care because HY spend less time in the nest, and also showed higher reretrievings and atypically retrievings respect to LY and SD dams. The aim of this study was determine if the poor sexual performance in HY rats is due to atypical maternal care. We used cross-fostering between HY and LY sublines and SD rats, in order to establish how maternal care affects male sexual behavior, we also measured the yawning frequency. Our results indicate that male rats HY raised by mothers LY decrease their yawning frequency until 7 ± 2.8 yawns per hour (y/h; $P < 0.05$), HY rats reared by their biological mothers yawned 25 ± 2.8 per hour, similar values were obtained when HY males were reared by SD dams reaching 17 ± 3.8 y/h ($P < 0.05$). In the first copulatory series, both groups of HY rats reared by LY and SD dams reach ejaculation in less than 30 min. However, LY rats reared by HY dams decreased their sexual performance because they never attempt to mount respect to LY reared their own mothers which were able to display mounts and intromissions. Using a binomial proportion inference the probability that HY dams reared offspring with higher incidence of NC and S are being significantly higher than LY and SD dams ($P<0.05$) In conclusión, HY dams phenotype impair sexual perfomance in their siblings independently of their origin.

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Poster

539. Sexual Behavior

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Title: Proceptivity differs in high-yawning respect to low-yawning and Sprague-Dawley rats

Authors: *A. MORA-BOLAÑOS, C. CORTES, J. R. EGUIBAR;
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Abstract: Receptive behavior in females is characterized by lordosis and proceptive behaviors consist in hopping, darting, and ear wiggling displays. Receptivity in female rats is stimulated during the estrous cycle due to sequential secretion of ovarian steroids. The female sexual behavior in ovariectomized rats can be induced by the sequential administration of estradiol and progesterone. Previous studies showed that high-yawning (HY) male rats had a deficient sexual performance when tested with outbred Sprague-Dawley (SD) female rats. The aim of the present study, was to analyze female sexual behavior display in rats selectively bred for HY and low-yawning (LY) spontaneous frequency, and outbred SD subjects tested in pairs with HY, LY and SD sexual experienced males. So, sexual receptivity was induced by sequential administration of estradiol benzoate (5 μg) and 44 h later by progesterone 2 mg diluted in olive oil and administered subcutaneously in 0.2 mL in the dorsal neck region. All subjects were tested 4h later with a sexual vigorous male of the same or different group of rats in circular Plexiglas arena (50 cm diameter). Our results showed that SD, HY and LY female rats showed lordosis quotients being 80 to 100% responsive to steroid treatment independently of the copulating male. However, HY female subjects showed less hopping behavior respect to LY and SD rats ($P<0.05$), but all groups of rats display similar amounts of darting's. In conclusion, only hopping differs among female groups tested suggesting that the neural circuits involve in hopping are less sensible to ovarian steroids in HY rats, but it is not the case with ventromedial hypothalamus that mediate lordosis which showed similar sensitivity to ovarian steroids in all subjects.

Disclosures: **A. Mora-Bolaños:** None. **C. Cortes:** None. **J.R. Eguibar:** None.

Poster

539. Sexual Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 539.08/FFF25

Topic: F.02. Behavioral Neuroendocrinology

Title: Gonadal steroids during puberty are necessary for steroid-independent male sexual behavior in castrated B6D2F1 hybrid male mice

Authors: *J. S. TEMPLIN, A. R. BARTLETT, A. BALA, A. L. ROKICKI, J. C. WYROSDIC, Z. Z. KHAN, J. PARK;
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Abstract: Gonadal steroids play an integral role in male sexual behavior (MSB), and in most rodent models, this relationship is tightly coupled. However, many other species, including humans, continue to demonstrate MSB in the absence of gonadal steroids, and the mechanisms that regulate steroid-independent MSB are not well understood. ~30% of castrated male B6D2F1 hybrid mice display MSB up to 26 weeks after castration, allowing for the investigation of individual variation in steroidal regulation of MSB. During both the perinatal and pubertal critical periods, the organizational effects of gonadal steroids on sexual differentiation of the neural circuits controlling MSB are well-documented. Several factors can alter the normal range of gonadal steroids which may lead to the disruption of the normal processes of masculinization and defeminization. It is unknown whether the organizational effects of gonadal hormones during puberty are necessary for steroid-independent MSB. Our results indicate that gonadal steroids during puberty are necessary for steroid-independent MSB in adulthood. Furthermore, gonadal steroids during puberty were not necessary for either testosterone (T) or estradiol to activate MSB in adulthood. Additionally, T has been correlated with reduced anxiety-like behavior in adult rodents, and prepubertal castration alters adult anxiety-like behavior. B6D2F1 hybrid male mice that were castrated prior to puberty displayed heightened anxiety-like behavior relative to controls, suggesting that testicular steroids during puberty are required for the normal organization and maturation of the developing stress axis. Anxiety-like behavior of B6D2F1 hybrid male mice, that were castrated in adulthood and expressed steroid-independent MSB, did not differ relative to those that ceased to express MSB. Furthermore, testosterone- and estradiol-treated males that were castrated prior to puberty exhibited less anxiety-like behavior relative to sham controls in adulthood, indicating that exposure to gonadal steroids during puberty was not necessary for the activation of the anxiolytic action of gonadal steroids. In summary, gonadal steroids during puberty are necessary for the normal maturation of neural circuitry that mediates steroid-independent MSB in adult castrated B6D2F1 male mice, but not necessary for the activation of MSB with T or estradiol. Whether the MSB that is activated with T or estradiol in hybrid males castrated prior to puberty differs from the steroid-activated MSB of males that were castrated in adulthood that do not demonstrate steroid-independent MSB warrants further investigation.

Disclosures: J.S. Templin: None. **A.R. Bartlett:** None. **A. Bala:** None. **A.L. Rokicki:** None. **J.C. Wyrosdic:** None. **Z.Z. Khan:** None. **J. Park:** None.

Poster

539. Sexual Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: CONACyT Grant 253631

Fronteras Grant 374

PAPIIT Grant 210215

Fellowship CONACyT 245202

Title: Survival of new neurons in the olfactory bulb induced by paced mating in the female rat

Authors: *R. A. ALVARADO, M. SANTOYO-ZEDILLO, R. G. PAREDES;
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Abstract: Previous research from our laboratory showed that when female rats pace the sexual interaction, the number of new cells increases in the accessory olfactory bulb (AOB) and the main olfactory bulb (MOB) 16 days after mating, in comparison to females that did not pace the sexual interaction. We also reported that repeated sessions of paced mating increases the number of new cells, compared to females that mated just once. The aim of present experiment was to evaluate if 10 paced mating sessions maintains this initial increase in the number of cells 45 days after mating and whether there are changes in the phenotype of these new cells. Female Wistar rats (220-250 grs), were ovariectomized and injected the first day of testing with DNA synthesis marker 5'-bromo-2`-deoxyuridine (BrdU, 300 mg / kg). After the last mating session subjects were anesthetized and sacrificed, their brain collected and placed in TBS with sucrose (at 30%) for protection against the cold. The brain was cut in sagittal sections (30 microns). For histological analysis we select those slices in which the AOB and MOB were present. For detection of the new cells with BrdU we used the chromogen solution nickel chloride-3,3'-diaminobenzidine (DAB). No differences were found in the number of cells between groups in the different layers of the OB, contrasting with what we have observed at day 16. Futures studies need to analyze the number of new neurons. Grants: CONACyT 253631, Fronteras 374; PAPIIT 210215. PhD. Fellowship CONACyT 245202

Disclosures: R.A. Alvarado: None. **M. Santoyo-Zedillo:** None. **R.G. Paredes:** None.

Poster

539. Sexual Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 539.10/GGG1

Topic: F.02. Behavioral Neuroendocrinology

Support: CONACYT 252756, 167101

Fronteras 374

UNAM-DGAPA-PAPPIT IN203615, IN210215

Title: Changes in c-fos expression patterns due to sexual experience in the female mice brain

Authors: *P. MARCO MANCLUS, W. PORTILLO, R. G. PAREDES;
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Abstract: In mice, unlike rats, naïve females showed low levels of sexual receptivity toward males in their first sexual experience. Female mice have a stereotypical posture called Lordosis, in which they flex their back exposing the genitalia and facilitating penetration. Using this reflex, sexual receptivity can be measured with the Lordosis Quotient (LQ, number of lordosis displayed/number of mounts). The activity of the brain cells displayed during a determined behavior can be evaluated by the detection of the proteins resulting of the product of the transduction of the "early expression genes" (genes expressed immediately after the activation of the cell).
The aim of this study is to evaluate if sexual experience induces plastic changes that involved the increase in cell activity in those areas related with the female sexual behavior such as: the Olfactory bulb, the Medial Preoptic Area, the Ventromedial Hypothalamus and the Bed nucleus of the Stria Terminalis.
In this study 54 CF1 female mice (Mus Musculus) were ovariectomized and randomly assigned to 1 of 3 groups: Experienced females (n=20), which received 6 mating sessions; Unexperienced females (n=18), which receive only one session; and Naïve females (n=18), which had no previous sexual experience. The mating sessions consist in placing the female in the male's cages for an hour, and during the test sexual behavior was registered. Females were hormonally primed with 1 µg of Estradiol and 100 µg of progesterone (48h and 4h respectively, before the test). All females receive 6 injections of each hormone to make groups comparable. Each group was divided in 3 other subgroups: Mating group, which receive a 7^{th} mating session; Olfaction group, which was exposed to bedding impregnated with male sexually relevant odors; and Control group, which were exposed to clean bedding. 90 min after the last test, animals were euthanized and perfused, and their brains were collected and sliced (20 µg thick). Brain sections were immunostained for C-fos, using Nissl technique as a counterstaining,

in order to evaluate the expression of this protein. Our behavioral results showed that, as expected, females increase their LQ with repeated testing, and our preliminary immunohistochemistry results suggest that females that mate 7 times have a higher number of C-fos immunoreactive cells in the ventromedial hypothalamus that those that mated twice, suggesting that sexual experience increases the number of cells that respond to mating stimulation.
This research was supported by grants CONACYT 252756, 167101; Fronteras 374; UNAM-DGAPA-PAPPIT IN203615, IN210215. We thank Francisco Camacho and Deisy Gasca for their technical assistance.

Disclosures: P. Marco Manclus: None. **W. Portillo:** None. **R.G. Paredes:** None.

Poster

539. Sexual Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 539.11/GGG2

Topic: F.02. Behavioral Neuroendocrinology

Support: Brinkman Family Foundation Grant

Title: The effects of intranasal oxytocin on anxiety, social, and sexual behaviors in male rats prenatally exposed to valproic acid

Authors: *S. M. HARDING[1], E. C. MASTERS[2];
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Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that affects 1 in 68 children, and is characterized by impairments in social behaviors and communication. In utero exposure to the antiepileptic drug valproic acid (VPA) during prenatal development is associated with ASD-like symptoms in children, making this a plausible rodent model. One potential treatment for ASD symptoms is the neuropeptide oxytocin (OXT), which contributes to social recognition, communication, and social bonding. Therefore, the present set of experiments was designed to examine whether intranasal administration of oxytocin would improve behaviors in male rats that were prenatally exposed to VPA. Pregnant Long-Evans female rats (n=3) were injected subcutaneously with either 600 mg/kg of VPA or saline on gestational day 12.5. After weaning, male pups were housed in same-sex conditions and received 0.8 IU/kg of intranasal OXT or saline daily from P21 to P41, forming three groups: VPA (VPA plus saline treatment, n=9), VPA-OXT (n=8), and Controls (saline plus saline treatment, n=4). Behavioral tests for anxiety, sociability, and sociosexual behaviors were conducted to investigate acute and long-

term effects of intranasal OXT treatment. On the elevated plus maze test, VPA rats showed an increased latency to enter the open arm compared to controls ($p<.05$), suggesting heightened anxiety. Likewise, during the sociability test, VPA rats spent more time in a neutral chamber than the Control rats ($p<.05$), suggesting they were less social. On both tests, the VPA-OXT group did not differ from Controls, providing evidence that OXT treatment may restore behaviors. To examine whether OXT treatment early in life had long-term effects on behavior, we tested all groups for social interactions with female rats in adulthood (P82-P98), well after OXT treatment had ceased. Sociosexual behaviors were assessed with tests for copulation, partner preference and ultrasonic vocalizations / scent marking. Surprisingly, early OXT treatment in VPA rats was associated with reduced sociosexual behaviors in adulthood. On copulation tests, the VPA-OXT group showed fewer mounts than both VPA and Control groups ($p<.01$). The VPA-OXT group also showed fewer scent marks than the VPA group, although the difference was did not reach statistical significance. Taken together, these findings have direct implications for the treatment of neurodevelopmental disorders like ASD with OXT. However, the long-term effects of early OXT exposure may require additional study.

Disclosures: S.M. Harding: None. **E.C. Masters:** None.

Poster

539. Sexual Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 539.12/GGG3

Topic: F.02. Behavioral Neuroendocrinology

Title: Motivational state, sexual experience and vaginal stimulations during mating influence Oxytocin

Authors: *M. L. LANGETT, N. M. CAMERON;
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Abstract: Various mating paradigms have shown to affect oxytocin (OT) neuron populations in the paraventricular nucleus (PVN) and the supraoptic nucleus (SON). However, the effect of various mating conditions on female plasma OT levels has not been fully investigated. Furthermore, the sexual experience state of the females is not consistent between experiments, making results difficult to interpret. We hypothesize that OT production and release are linked to the quality of sexual experience, the physical stimulations received and the motivational state of the female. We designed two studies to test this hypothesis. In Experiment 1, the effect of mating experience in ovariectomized (ovx) steroid-primed females on OT levels was investigated under paced mating conditions. We found a significant positive correlation in the virgin females

between plasma OT levels and para-copulatory behaviors (hopping, darting and ear wiggling) that was not observed in sexually- experienced females. We also found no significant effects of sexual experience on OT levels. In Experiment 2, sexually-experienced ovx females were used to investigate the effects of vaginal cervical stimulation (VCS) and paced mating on brain and plasma OT levels. These females were placed in four mating conditions (either paced or not paced, and with or without vaginal mask). Across the four groups, there was a significant negative correlation between PVN OT levels and para-copulatory behaviors. In animals that received VCS, a significant positive correlation between plasma OT levels and para-copulatory behaviors was found. There was a significant positive correlation between PVN and SON OT levels in females that mated but did not receive VCS. Additionally, experienced females that received VCS and were paced mated showed a significant negative correlation between PVN and plasma OT levels. In both of these experiments, brain and plasma OT was collected and analyzed using an ELISA. Overall, these results indicate the importance of mating conditions and sexual experience on OT production and release. Para-copulatory behaviors, which are voluntary behaviors that could reflect the motivational state of females, seem to be particularly important in the modulation of OT levels, decreasing it in the PVN and increasing it in the plasma. This suggests a relationship between PVN and plasma OT levels. Interestingly PVN and SON levels correlated only when females did not receive VCS, further suggesting a link between sexual experience and brain OT release. In conclusion, motivational state, sexual experience and vaginal stimulations during mating may influence production and release of OT after mating in the rat.

Disclosures: M.L. Langett: None. **N.M. Cameron:** None.

Poster

539. Sexual Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 539.13/GGG4

Topic: F.02. Behavioral Neuroendocrinology

Support: Canadian Institutes of Health Research (CIHR)

Title: Getting real: Oxytocin facilitates goal-tracking to a real sex partner relative to a distal second-order sexual cue in male rats

Authors: *L. M. SPARKS, V. PALLIKARAS, J. G. PFAUS;
Ctr. for Studies in Behavioral Neurobio., Concordia Univ., Montreal, QC, Canada

Abstract: Repeated pairings of a conditioned stimulus (CS) with an unconditioned stimulus (US) result in individual differences in Pavlovian-conditioned approach (PCA) toward the CS (sign-tracking; ST) or US (goal-tracking; GT) using sexual reward. Furthermore, a subset of animals termed intermediates (IN) exhibit PCA toward both the CS and US equally. Oxytocin (OT), a neuropeptide involved in sexual arousal and social bonding, enhances the acquisition of conditioned ejaculatory preference (CEP) for olfactory stimuli. Whether OT can potentiate cue- or goal-directed behaviour through the development of CEP in rats identified as ST, GT or IN has yet to be elucidated. ***Objective***: The present study examined whether OT alters the expression of a CEP toward a visuo-tactile cue in ST, GT and IN male rats. ***Method***: Sexually-naïve, male Long-Evans rats received 13 Pavlovian conditioning sessions in an individualized compartment of an open field chamber, where an orange cone CS (2-min/presentation) predicted the opportunity to copulate to ejaculation in a separate compartment with a receptive female (US). On sessions 7-13, rats received injections of saline or OT (5µg/ml/kg; s.c.) 4-h, 2-h, and 15-min prior to the start of the trial. Pavlovian-conditioned approach toward the CS (ST) and US (GT) was measured by the proportion of time spent in an area centered around the CS or the door to the female compartment, respectively, in the absence and presence of the cue. ***Preliminary Results***: Between sessions 1 and 7, rats did not display ST or GT behaviour; no differences were observed in PCA toward the CS- or US-designated areas. By session 13, individual differences in PCA were observed, where a subset of rats spent a significantly greater proportion of time in either the CS- (ST) or US-designated (GT) areas. Interestingly, OT did not facilitate the development of ST or GT behaviour in animals displaying such phenotypes. However, in IN animals, OT shifted PCA toward the US in both the presence and absence of the cue. ***Conclusions***: These results provide further evidence that conditioned cues acquire incentive motivational properties through Pavlovian conditioning with sexual reward as the US. Moreover, our preliminary results suggest OT may be important in the formation of Pavlovian associations between the CS and US, and may guide IN rats toward a GT behavioral phenotype.

Disclosures: **L.M. Sparks:** None. **V. Pallikaras:** None. **J.G. Pfaus:** None.

Poster

539. Sexual Behavior

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 539.14/GGG5

Topic: G.02. Motivation

Support: NSERC

Title: Ovarian hormones alter the expression of 50 kHz ultrasonic vocalizations induced by distributed clitoral stimulation in female rats

Authors: *C. A. GERSON[1], T. SCARDACHIO[1,2], G. R. QUINTANA[1], P. B. S. CLARKE[2], J. G. PFAUS[1];
[1]Psychology, Concordia Univ., Montreal, QC, Canada; [2]Pharmacol., McGill Univ., Montreal, QC, Canada

Abstract: Adult rats emit frequency modulated 50kHz ultrasonic vocalizations (USVs) in response to rewarding stimuli. Male rats, for example, emit these calls as they are about to ejaculate. Investigations of the incentive-reward value of female vocalizations during sexual interaction with either devocalized or vocally-intact males suggest that female USVs are activated by ovarian hormones and the presence of a male overall, and are not correlated with copulatory stimulation. We have shown previously that artificial distributed clitoral stimulation (CLS) that mimics the tactile CLS experienced by the female during male mounts with pelvic thrusting induces a sexual reward state capable of supporting both conditioned place and partner preferences. The present study examined whether distributed CLS might induce specific frequency-modulated calls in female rats, and whether the calls would be altered by different hormone priming. Virgin female rats were ovariectomized and injected with either estradiol benzoate (EB) and progesterone (P), EB alone, P-alone, or the oil vehicle, prior to the administration of distributed CLS. CLS was made by lightly brushing the clitoris in a downward stroke using a No. 4 paintbrush. CLS was applied every 5 sec for 1 min after a 4 min baseline USV measurement and this was repeated for 6 cycles over a period of 34 min, for a total of 72 CLSs. USVs were recorded and analyzed before, during, and after each bout of CLS. Females primed with EB+P displayed a large number of 50 kHz-range trills and flats that were long in duration and primarily of the trill subtype. Females primed with EB-alone emitted a moderate number of 50-kHz calls during CLS, whereas females primed with P-alone emitted few calls during CLS. Unprimed females rarely called, and those that were emitted were of very short duration and usually of the flat subtype. Thus, in contast with previous reports, we show that female rats emit a consistent pattern of 50 kHz calls in response to rewarding stimulation of the clitoris, and that the sensitivity to CLS in the induction of these calls is augmented by steroid hormones. Supported by NSERC. *Keywords: Vocalization, Clitoral Stimulation, Ovarian Hormones, Sexual Reward*

Disclosures: C.A. Gerson: None. **T. Scardachio:** None. **G.R. Quintana:** None. **P.B.S. Clarke:** None. **J.G. Pfaus:** None.

Poster

540. Stress: Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 540.01/GGG6

Topic: F.04. Stress and the Brain

Support: CIHR

NSERC

Title: Inhibition of anterior dorsomedial accumbens shell neurons prevents stress-induced decrease in sucrose intake.

Authors: *A. MITRA[1], J. SEIGNEUR[2], G. GUEVREMONT[1], I. TIMOFEEV[2], E. TIMOFEEVA[1];
[1]IUCPQ, Univ. Laval, Quebec, QC, Canada; [2]IUSMQ, Univ. Laval, Quebec, QC, Canada

Abstract: Introduction: Accumbens shell is a reward and stress responsive area of the brain. It's pharmacological and optogenetic inhibition leads to increased food intake whereas its specific temporal activation terminates the ongoing feeding bout. Acute stress leads to anorexic response towards normal and palatable food. We hypothesized that stress activates the accumbens shell neurons, hence leading to decreased sucrose consumption; and thus, its inhibition following acute stress could rescue the stress-induced anorexia. **Method**: Male Sprague Dawley rats were implanted with metal microelectrodes in the anterior dorsomedial accumbens shell (admAcbSh) and extracellular recordings performed during 1 h of 10% sucrose solution access, with or without foot-shock stress. Electrophysiological parameters were used to characterize neurons into putative medium spiny GABAergic neurons (pMSN), putative fast-spiking GABAergic interneurons (pFSI) and putative tonically-active cholinergic interneurons (pTAI). Firing rate changes of these putative neurons were studied around the sucrose licking clusters. Separate cohorts of rats were microinjected with channelrhodopsin (ChR2), halorhodopsin (eNpHr) or control non-opsin (eYFP) viral constructs in admAcbSh followed by implantation of fiber-optic cannula in the same region. Effect on sucrose intake and general anxiety status was tested using various optical parameters during non-stressed and stressed conditions. To validate the in-vivo optical stimulation parameters, in-vitro whole cell recordings were performed. **Results**: Acute stress decreased sucrose intake by modulating lick microstructure. The admAcbSh had higher percentage of pMSN population which showed increased firing rate during sucrose intake following stress. Specifically, it was the cluster-start excited neurons that increased in percentage and firing rate following stress. Optogenetic activation of the admAcbSh led to a significant decrease in sucrose consumption by decreasing motivational value of sucrose. Conversely, optogenetic inhibition of the admAcbSh attenuated stress-induced anxiety and increased sucrose intake in both experimental conditions. Patched

pMSN neurons expressing ChR2 or eNpHr show robust excitatory or inhibitory responses, respectively, when optically stimulated. **Conclusion**: Increase in firing rate of the admAcbSh neurons induced anorexia towards palatable food, and inhibition of admAcbSh reinstates the normal eating behavior in stressed rats. Specific modulation of this structure could therefore be used to prevent stress-induced ill-metabolic consequences of eating disorders.

Disclosures: A. Mitra: None. **J. Seigneur:** None. **G. Guevremont:** None. **I. Timofeev:** None. **E. Timofeeva:** None.

Poster

540. Stress: Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 540.02/GGG7

Topic: F.04. Stress and the Brain

Support: TCU/RCAF # 33503

MEC PSI-2013-44945-P

Title: Dorsomedial striatum lesions affect adjustment to reward uncertainty, but not to reward devaluation or omission

Authors: *M. R. PAPINI[1], A. C. GLUECK[1], S. E. CONRAD[1], I. MORON[2], C. TORRES[3]; [1]Dept of Psychology, Texas Christian Univ., Fort Worth, TX; [2]Univ. of Granada, Granada, Spain; [3]Univ. of Jaen, Jaen, Spain

Abstract: The proposal suggesting that the dorsomedial striatum (DMS) is implicated in the acquisition of reward representations leads to the hypothesis that it should play a role in situations involving reward loss. The current experiment explored the effects of DMS excitotoxic lesions on consummatory successive negative contrast (cSNC; reward devaluation), autoshaping training with partial vs. continuous reinforcement (PR, CR; reward uncertainty), and appetitive extinction (reward omission). For reward devaluation testing, animals having access to 32% sucrose for 5 min during 10 daily sessions were unexpectedly downshifted in 5 subsequent sessions to 4% sucrose. Their consummatory behavior was compared to that of animals that received access to 4% sucrose during each of 15 sessions. Animals with DMS lesions exhibited a normal cSNC effect, showing significant consummatory suppression during downshift sessions relative to unshifted controls. For reward uncertainty testing, animals received lever-pellet Pavlovian pairings with either 50% PR or 100% CR. Sham animals displayed higher lever-pressing performance under PR than under CR training (the PR acquisition effect), whereas this

effect was eliminated by the DMS lesion. Interestingly, goal entries during acquisition were also affected by the DMS lesion, but in the opposite direction relative to lever pressing. Despite its effects on acquisition, DMS animals showed unaffected extinction of lever pressing and goal entries. Finally, open field testing indicated normal motor behavior. Thus, DMS lesions selectively affected the behavioral adjustment to a situation involving reward uncertainty, producing a behavioral reorganization according to which goal tracking (goal entries) become predominant at the expense of sign tracking (lever pressing). These results suggest that the function of the DMS in situations involving reward loss is not general, but restricted to reward uncertainty. We suggest that a nonassociative, drive-related process induced by reward uncertainty requires normal output from DMS neurons.

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Poster

540. Stress: Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 540.03/GGG8

Topic: F.04. Stress and the Brain

Title: Changes in apoptotic factors in the nucleus accumbens on morphine-induced conditioned place preference in the rat: involvement of acute and subchronic physical stress

Authors: *Y. RAZAVI[1], A. HAGHPARAST[2];
[1]Cell. and Mol. Res. Ctr., Tehran, Iran, Islamic Republic of; [2]Shahid Beheshti Univ. of Med. Sci., Neurosci. Res. Ctr., Tehran, Iran, Islamic Republic of

Abstract: Introduction: It has been shown that nucleus accumbens (NAc) has an essential role in rewarding action of opiates and is also influenced by stress. Additionally, morphine and stress both can induce apoptosis in different kinds of cells such as neural cells. In this study, we have investigated the effects of morphine-induced conditioned place preference (CPP) in presence and absence of physical stress on changes in conditioning score and the level of apoptotic factors (Bax/Bcl-2 ratio, caspase-3 activation and PARP degradation) in the NAc **Material and Methods:** Male Wistar rats were divided to two saline- and morphine-treated supergroups. Each supergroup consisted of control, acute stress (AS, received forced swim stress just one day) and subchronic stress (SS, received forced swim stress for three consecutive days) groups. In all of groups, the CPP paradigm was done and following behavioral experiments, the alternation of apoptotic factors in the NAc were measured by western blot analysis. **Results:** The results indicated that in saline or morphine-treated animals, AS and SS could enhance Bax/Bcl-2 ratio,

caspase-3 activation and PARP degradation in the NAc. **Conclusion:** In the present study, These finding demonstrated that both AS and SS trigger apoptotic events in saline or morphine-treated animals as well as these effects in morphine-treated animals were more considerable than those of saline-treated animals.

Disclosures: Y. Razavi: None. **A. Haghparast:** None.

Poster

540. Stress: Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 540.04/GGG9

Topic: F.04. Stress and the Brain

Title: Examination of cortizol receptor and endogenous opioid expression in a primate model of self injurious behavior

Authors: *M. JACKSON[1], B. FORET[2], J. FONTENOT[3], E. ROMERO[3], D. HASSELSCHWERT[3], J. SMITH[3], K. SMITH[2];
[1]Biol., Univ. of Louisiana At Lafayette, Lafayette, LA; [2]Univ. of Louisiana at Lafayette, Lafayette, LA; [3]New Iberia Res. Ctr., New Iberia, LA

Abstract: Suicide is the tenth leading cause of death in the United States, the second leading cause of death for those between the ages of 15 to 24, and accounted for approximately 41,000 deaths in the year 2014. According to the Centers for Disease Control, the suicide rate has increased a dramatic 24% in 15 years. With such a prevalent impact on our society, it is important to understand the underlying mechanisms that may lead a person to self harm. Non-suicidal self-injury (NSSI) is the deliberate infliction of physical harm to one's own body without suicidal intent. While NSSI is by definition, not an attempt at suicide, it is strongly associated with future suicide ideation and has been described as a "gateway" behavior. Self-injurious behavior occurs in approximately 1-4% of the adult human population, with higher rates of incidence in the adolescent and institutionalized populations. SIB also occurs in a low percentage of captive monkeys; it is an endogenously occurring model of SIB in humans in the context of captive animals. Rhesus Macaque monkeys are evolutionarily and physiologically similar to humans, share 93% of their DNA with humans and consequently have long been used as testing subjects for vaccine and clinical trials. In order to study SIB we used 8 sex-matched pairs of rhesus macaques, eight who exhibited self-injurious behavior (SIB) and eight controls, to examine alterations in gene expression. The brain regions chosen are those closely linked to reward reinforcement and stress adaptation including the hypothalamus, orbital frontal cortex, nucleus accumbens, hippocampus, caudate, putamen and the amygdala. Previous studies have

identified reactive changes in astrocytes of animals exhibiting SIB. We have therefore collected five additional rhesus macaques, two who exhibited SIB, to examine morphological changes in astrocytes. Thus far, our findings suggest no significant changes in the gene expression of the mu-opioid receptor, mineralocorticoid receptor, glucocorticoid receptor, β-endorphin precursor molecule proopiomelanocortin (POMC), or the dynorphin A and B precursor prodynorphin in the hypothalamus, hippocampus and orbital frontal cortex. Current studies are investigating the expression levels of these genes in the nucleus accumbens, caudate, putamen, and amygdala. Previous studies have hypothesized that altered endogenous opioid expression occurs in the brains of individuals and animals that self-injure. Our studies indicate the need for further studies to identify if, and how this is occurring in self-injuring macaques.

Disclosures: **M. Jackson:** None. **B. Foret:** None. **J. Fontenot:** None. **E. Romero:** None. **D. Hasselschwert:** None. **J. Smith:** None. **K. Smith:** None.

Poster

540. Stress: Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 540.05/GGG10

Topic: F.04. Stress and the Brain

Support: NARSAD YI Award

NIH Grant MH086828

Title: Chronic stress alters the electrophysiological properties of hippocampal-accumbens synapses

Authors: *T. A. LEGATES, S. M. THOMPSON;
Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Depression is a leading cause of disability with a lifetime prevalence of 12% and 20% in men and women, respectively. A common risk factor that increases the likelihood of depressive episodes is stress. Depressed patients report more stressful life events than non-depressed subjects. The nature of the stress-induced neuronal changes that promote depression in susceptible individuals remains unknown. There is increasing evidence that chronic stress weakens neuronal communication in multiple brain regions, perhaps accounting for the wide range of behaviors affected by stress. Previous work has focused on the changes that occur *within* specific brain regions, such as the hippocampus and nucleus accumbens (NAc). However, communication *between* these two areas is important as well, yet poorly understood. The ventral

hippocampus provides excitatory input to the NAc shell, which is thought to be important for modulating NAc activity and providing contextual information to reward processing. This synapse has received increased attention due to its potential role in mood regulation, specifically in response to reward and motivation to seek rewards, which are altered in mood disorders including depression. We used whole-cell electrophysiological recordings in the NAc shell to characterize the properties of the connections from the hippocampus to the NAc in the brains of mice, including the contributions of glutamate receptor subtypes. We found that Hip-NAc synapses become strengthened in response to high frequency activity due to classical activity-dependent long-term plasticity mechanisms but independent of dopamine receptor signaling. Furthermore, we found that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit composition remains unchanged after LTP induction. These results were similar in D1- and D2-MSNs. We then used a chronic stress paradigm to induce depression-like behavior. We found that the properties of this synapse change in response to chronic stress. However, the observed changes differ in D1- and D2-MSNs. This work defines a specified neuronal circuit that is responsible for mood regulation and further our understanding of excitatory synaptic strength as a critical mediator of this process. Understanding the neuronal changes that underlie depression and antidepressant response will provide key insight into developing new, more effective treatments for this disorder.

Disclosures: **T.A. LeGates:** None. **S.M. Thompson:** None.

Poster

540. Stress: Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 540.06/GGG11

Topic: F.04. Stress and the Brain

Support: ALW

Title: Circadian glucocorticoid oscillations determine activity in the basolateral amygdala

Authors: F. S. DEN BOON[1], M. J. N. BAELDE[1], *M. JOELS[2], H. KARST[1];
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Abstract: The hypothalamic-pituitary-adrenal (HPA) axis is a key part of the stress system. The HPA axis regulates plasma glucocorticoid levels and thereby controls the rapid response to stress. Under basal conditions, the synthesis and release of glucocorticoids occur in a circadian rhythm overarching ultradian pulses. Peak levels of glucocorticoids are seen at the start of the active phase, which is nighttime in most rodents. Deregulation of the HPA-axis can result in a

wide range of disorders, including mood disorders. Limbic areas express high levels of receptors sensitive to corticosteroids (CORT) and are especially affected by changes in CORT levels. We demonstrated earlier that repeated application of CORT results in immediate and long-lasting changes in spontaneous glutamate transmission in the basolateral amygdala (BLA), an area that regulates fear behavior. To study the effect of circadian fluctuations of CORT levels on glutamatergic neurotransmission in the BLA, we investigated spontaneous glutamate release in BLA slices of mice sacrificed at the start of the inactive phase and at the start of the active phase. To mimic in vivo exposure to ultradian glucocorticoid pulses, 'inactive phase slices' were exposed to increasing concentrations of CORT (3, 10, 30 and 100 nM, 1 hr between pulses). Active phase slices were exposed to decreasing concentrations of CORT (100, 30, 10 and 3 nM, 1 hr between pulses). Miniature excitatory postsynaptic currents (mEPSCs) were recorded in the presence of TTX and bicuculline. Results showed that the basal mEPSC frequency was increased in inactive phase slices over the course of the experiment (1.11±0.14 Hz, 2.28±0.38 Hz, $p<0.05$). The basal mEPSC frequency was reduced in active phase slices (1.63±0.20 Hz, 0.91±0.12 Hz, $p<0.05$). Furthermore, tone-cued fear conditioning experiments with similar groups of animals showed that mice at the start of the inactive phase show more freezing behavior, a measure of fear, than mice at the start of the active phase (63.92±3.18%, 38.46±4.53%, $p<0.05$). These data indicate that neuronal activity in the BLA is controlled by the pulsatile circadian rhythm in which glucocorticoids are released.

Disclosures: **F.S. den Boon:** None. **M.J.N. Baelde:** None. **M. Joels:** None. **H. Karst:** None.

Poster

540. Stress: Neural Circuits

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Topic: F.04. Stress and the Brain

Support: NSF Grant 1257284

Hartwell Individual Biomedical Research Award

NARSAD Young Investigator Award

Title: Investigating frontostriatal circuit function in social interaction behavior and chronic social stress

Authors: ***B. S. HALL**, R. N. FETCHO, T. HUYNH, A. RAJADHYAKSHA, C. LISTON; Weill Cornell Med. Col., New York, NY

Abstract: Impairments in social functioning are a core component of many stress-related neuropsychiatric conditions including depression, schizophrenia and anxiety disorders. The underlying mechanisms that lead to social dysfunction in these conditions are not well understood, but are thought to involve the nucleus accumbens (NAc), a stress-sensitive area of the ventral striatum that regulates drives and motivation. The NAc integrates signals from a reward-processing network that includes the infralimbic (IL) region of the prefrontal cortex. The mechanisms by which IL-to-NAc projections influence social behavior and how they are altered by chronic stress have not been well defined. This work aims to investigate how projections from the IL modulate activity in the NAc and influence social interaction behavior in order to define circuit mechanisms by which these processes are altered by chronic stress. Using a rodent model of chronic stress (chronic social defeat stress) and fiber photometry in order to record from and manipulate specific neural populations and circuitry, we show how chronic stress affects NAc activity and IL-to-NAc signaling in the context of social interaction behavior. This work may elucidate mechanisms by which stress can lead to changes in reward circuitry that impact social interaction and lead to social dysfunction in psychiatric diseases.

Disclosures: B.S. Hall: None. **R.N. Fetcho:** None. **T. Huynh:** None. **A. Rajadhyaksha:** None. **C. Liston:** None.

Poster

540. Stress: Neural Circuits

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Topic: F.04. Stress and the Brain

Support: NIH U01 AA013641

NSF Graduate Research Fellowship F031543

Title: Localization of CRF-binding protein in GABAergic interneurons in the prefrontal cortex

Authors: *K. KETCHESIN, A. SEASHOLTZ;
Univ. of Michigan, Ann Arbor, MI

Abstract: Corticotropin releasing factor (CRF) is the key central nervous system regulator of the mammalian stress response. CRF mediates its primary effects through binding to CRF receptors, and dysregulation of the CRF system has been linked to alcohol addiction. The activity of CRF is also modulated by the CRF-binding protein (CRF-BP), a 37 kDa secreted glycoprotein that binds CRF with very high affinity and regulates CRF receptor activation. We have recently shown that

CRF-BP mRNA expression is significantly decreased in the medial prefrontal cortex (mPFC) after repeated cycles of binge drinking in mice. However, the neurotransmitter/neuropeptide phenotype of CRF-BP-expressing cells in the PFC is currently unknown. The goal of the current study was to characterize the expression of CRF-BP in excitatory and inhibitory neurons of the PFC. Dual in situ hybridization experiments were performed to determine the colocalization of CRF-BP mRNA expression with vesicular glutamate transporter (VGLUT) mRNA and glutamate decarboxylase (GAD) mRNA in the PFC of male C57BL/6 mice. CRF-BP mRNA was highly colocalized with GAD, but not VGLUT, in the PFC, suggesting a role in inhibitory GABA neurons. To further characterize the specific GABAergic interneuron subtype, dual in situ hybridization experiments were performed for CRF-BP mRNA and various neuropeptides, including cholecystokinin (CCK), somatostatin (SST), vasoactive intestinal peptide (VIP), and parvalbumin. There was a high degree of colocalization of CRF-BP with parvalbumin and SST in the PFC, while the degree of colocalization of CRF-BP with VIP and CCK was significantly lower. Overall, these results suggest that CRF-BP is primarily expressed in GABAergic interneurons of the PFC, specifically in parvalbumin and SST-expressing neurons. Previous studies suggest that CRF is primarily expressed in GABAergic interneurons, while CRF-R1 is mainly expressed in excitatory pyramidal neurons of the cortex. The expression patterns observed in the current study suggest that CRF-BP may be positioned to act locally in interneurons of the mPFC to regulate CRF receptor activity on pyramidal neurons, perhaps modulating the output of these neurons. Ongoing studies are investigating the colocalization of CRF-BP with CRF in the PFC. Together, these colocalization studies will provide a more comprehensive understanding of the role of CRF-BP in regulation of CRF activity within the circuits of the stress and reward systems.

Disclosures: K. Ketchesin: None. **A. Seasholtz:** None.

Poster

540. Stress: Neural Circuits

Location: Halls B-H

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Program#/Poster#: 540.09/GGG14

Topic: F.04. Stress and the Brain

Title: Subchronic heat stress exposure increases activity in hippocampus and amygdala reflected as anxiety-like behavior of young Wistar rats

Authors: J. C. AVILA SANDOVAL, L. V. CONTRERAS, E. U. FRANCO ANDRADE, *J. GUZMÁN MUÑIZ, O. P. GONZALEZ-PEREZ, N. A. MOY LÓPEZ;
Psychology/Lab Neurosci., Univ. of Colima, Colima, México, Mexico

Abstract: Climate change is a current topic of research not only by their environmental effects, but also to the possible influence of high environmental temperatures in humans, which have been related to anxious and violent behavior, suggesting an existent biological factor between heat-anxiety and the stress-modulated structures. However, the relationship between anxiety and hot weather is not fully understood, which leaded us to examine the effects of subchronic heat stress exposure in anxiety-like behavior and amygdala/hippocampus activity in young Wistar rats. In order to accomplish this aim, 30 days old experimental rats (10 males and 10 females) were exposed to heat (33±3°C) for 6 hours during 7 days, whereas control group (10 males and 10 females) in standard laboratory conditions (24±1°C), all of them was kept under 12:12h light/dark cycle with water and food *ad libitum*. Anxiety index and motor activity were evaluated with Elevated Plus Maze and Open Field test respectively, while (Heat Shock Protein 70) HSP70 *and c-fos* markers were quantified by immunohistochemistry in amygdala (central and medial) and hippocampus (CA2, CA3 and DG). Behavioral results showed an increment in anxiety index and motor activity only in experimental males ($t=2.71$; $df=18$; $p=0.01$). On the other hand, a significant expression of *c-fos* protein on CA2, CA3, DG and medial amygdala was found between groups ($t=8.69$; $df=48$; $p=<0.001$). Nevertheless, a marked difference in staining was detected only in female CA2 ($t=2.58$; $df=48$; $p=0.01$) where experimental females had less expression of HSP70 than control females; while experimental males also showed a decreased expression of HSP70, but only in CA3 and DG hipocampal areas ($t=2.58$; $df=48$; $p=0.01$). This findings suggest that heat stress exposure in early ages can lead to sex dependent modifications in *c-fos* and HSP70 expression in amygdala and hippocampus, which leads to anxiety circuitry changes and it could reflect as aggressive behavior and irritability in humans.

Disclosures: J.C. Avila Sandoval: None. **L.V. Contreras:** None. **E.U. Franco Andrade:** None. **J. Guzmán Muñiz:** None. **O.P. Gonzalez-Perez:** None. **N.A. Moy López:** None.

Poster

540. Stress: Neural Circuits

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Program#/Poster#: 540.10/GGG15

Topic: F.04. Stress and the Brain

Support: NIH RO1 MH098348

Title: Amygdala and prefrontal cortex activity varies with individual differences in the stress response

Authors: *T. R. OREM, M. D. WHEELOCK, N. G. HARNETT, K. H. WOOD, A. M. GOODMAN, S. MRUG, D. C. KNIGHT;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Introduction
Prior work has shown that the prefrontal cortex (PFC) and amygdala mediate the stress response (Ressler 2010; Quirk and Beer, 2006). However, the neural substrates that underlie individual variability in the emotional response to stress have not yet been determined. The present study examined the relationship between PFC – amygdala circuitry and the psychophysiological response (e.g. heart rate and skin conductance) to stress.
Methods
Heart rate (HR), skin conductance response (SCR), cortisol, and blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) were measured while participants completed the Montreal Imaging Stress Task (MIST). The MIST is a well-validated psychosocial stress task that includes Stress and Control conditions. HR, SCR, and cortisol were monitored as indices of the peripheral emotional response to stress.
Statistical Analyses
To assess the relationship between brain activity and psychophysiological reactivity, the differences between Control and Stress MIST conditions was calculated for HR and SCR. The difference between baseline and post-stress tasks was calculated for cortisol. These measures were then regressed on BOLD fMRI data using the AFNI software package (Cox, 1996).
Results
HR and SCR were greater during the Stress than Control condition, indicating a differential emotional response to stress was evoked by the task. Cortisol levels were higher at baseline than post-task and were therefore inconsistent with the typical cortisol response to stress. Differential BOLD fMRI was observed within the ventral PFC and amygdala. Further, differential activity within these brain regions varied with the peripheral emotional response (i.e. HR and SCR) to stress.
Discussion
These results are consistent with the view the ventral PFC and amygdala support processes that are important for the expression and regulation of emotion. Findings from the present study suggest that psychosocial stress affects PFC-amygdala circuitry, controls the peripheral expression of emotion, and may account for individual variability in the stress response.
This research was funded by NIH RO1 MH098348 (Mrug and Knight).

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Poster

540. Stress: Neural Circuits

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: F.04. Stress and the Brain

Support: Indiana University

Title: Lasting sex differences in chronic stress-induced patterns of fos and brain-derived neurotrophic factor expression in prelimbic cortex in response to a novel acute stressor

Authors: *K. M. MOENCH, C. L. WELLMAN;
Dept. of Psychological and Brain Science, Program in Neurosci., Indiana Univ., Bloomington, IN

Abstract: Stress-related psychopathologies such as depression are associated with prefrontal cortex dysfunction, and are twice as likely to be diagnosed in women than men. Risk for stress-related disorders increases with the number of prior stressful life events. However, the neurobiological basis for increased vulnerability to multiple stressors in males and females has yet to be established. One possibility is that long-lasting alterations in factors that modulate prefrontal cortex activity, such as brain-derived neurotrophic factor (BDNF), could give rise to aberrant behavioral and neuroendocrine responses to subsequent stressors. Thus, we examined activity (via fos expression) and BDNF expression in prelimbic cortex (PL) of chronically stressed male and female rats in response to a novel stressor. Adult rats underwent chronic restraint stress (3h/day for 10 days) or were left unstressed (No Stress). Either one (Stressed) or 7 days later (Stress-Recovery), rats were exposed to a 30-minute elevated platform stressor. Rats were euthanized ~60 minutes after the cessation of the elevated platform stressor, and brains were removed and stained for fos and BDNF. Stereological estimates of fos-positive cells and relative luminosities of BDNF-expressing neurons were obtained for superficial and deep layers of PL. In response to a novel stressor, Stressed males had a decrease in fos expression in the superficial layers of PL compared to No Stress males, whereas Stressed females had increased fos expression throughout PL. For Stress-Recovery males, exposure to a novel stressor increased fos expression in the deep layers of PL, whereas fos expression in Stress-Recovery females was similar to No Stress females. Further, BDNF expression was increased in Stressed males, but not in Stress-Recovery males. In contrast, Stressed females showed little change in BDNF expression, whereas Stress-Recovery females showed an increase in expression. Thus, exposure to chronic stress alters the response of prelimbic cortex to novel stressors in in a sex-specific manner, and alterations in BDNF may contribute to this effect. Such chronic stress-induced changes in responses to subsequent stressors may contribute to the differential vulnerability of males and females to stress sensitive disorders.

Disclosures: **K.M. Moench:** None. **C.L. Wellman:** None.

Poster

540. Stress: Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 540.12/GGG17

Topic: F.04. Stress and the Brain

Support: MH049698

Title: Targeting glucocorticoid receptor deletion to Dlx 5/6 interneurons results in impulsive behavior and a higher peak corticosterone release in female, but not male, mice

Authors: ***J. SCHEIMANN**, P. MAHBOD, R. L. MORANO, M. FITZGERALD, B. PACKARD, J. P. HERMAN;
Univ. of Cincinnati, Cincinnati, OH

Abstract: Dysfunction in the activation or cessation of the hypothalamic-pituitary-adrenocortical (HPA) axis stress response is linked to stress-related affective disorders. The glucocorticoid receptor (GR) is the target of corticosterone released as part of the HPA axis stress response, and inappropriate signaling by this receptor is believed to play a role in stress-related pathologies. Glucocorticoid receptors in the forebrain, including the PFC, have been shown to be integral for cessation of the HPA axis response. Our group has demonstrated that chronic stress depletes GR in the PFC, largely targeting interneurons. In these studies, we tested the role of forebrain interneurons in regulation of PFC-related behaviors. The DLX 5/6 homeobox genes are expressed in developing and mature interneurons that arise from the medial ganglionic eminence, and migrate to the forebrain in areas such as the cortex, prefrontal cortex, striatum, and hippocampus. In this experiment, we generated interneuron-specific GR knockout mice by breeding GRflox mice with transgenic mice overexpressing Cre recombinase behind a DLX5/6 promoter. Adult female DLX 5/6 Cre+: GR f/f mice had a higher peak corticosterone release after 30 minutes of acute restraint and showed a reduced latency of escape behavior in a test of behavioral inhibition (passive avoidance). DLX 5/6 Cre+: GR f/f females did not exhibit demonstrable deficits in forced swim test or open field. In contrast, adult male DLX 5/6 Cre+:GR f/f mice did not show any behavioral or hormonal phenotype. This study supports the crucial role of glucocorticoid receptor on interneurons in forebrain for control of the HPA axis, and suggests that deletion of the receptor in these regions may impair behavioral inhibition and HPA axis control. This study also suggest that males and females may develop different mechanisms for control of the HPA axis and behavior following genetic deletion of GR in forebrain interneurons. Supported by MH049698.

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Poster

540. Stress: Neural Circuits

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Program#/Poster#: 540.13/GGG18

Topic: F.04. Stress and the Brain

Title: Learned helplessness induced by maternal separation of an animal model of posttraumatic stress disorder using a shuttle box in rats and levels of brain-derived neurotrophic factor in the hippocampus and the medial prefrontal cortex

Authors: ***M. TANICHI**, H. TODA, S. ENOMOTO, K. SHIMIZU, M. NAGAMINE, M. UENOYAMA, M. NIBUYA, A. YOSHINO;
Natl. Def. Med. Col., Saitama, Japan

Abstract: OBJECTIVES: We have reported posttraumatic stress disorder (PTSD) model of rat using a shuttle box, in which rats exposed to inescapable footshocks (IS) corresponding to trauma 2 weeks before had the persistent behavioral alterations characterized by hypoactive and hyperarousal behaviors. Clinical studies have previously shown that exposure to stress during the postnatal development periods is associated with an elevated risk for PTSD. But we reported last year in this congress that maternal separation (MS), which is one of the most commonly used procedures for inducing the early life stress in rodents, increased learned helplessness (LH) behaviors of our PTSD model rats using a shuttle box, not PTSD like behaviors. In this study, we examined the influence of MS on our PTSD model regarding of the mRNA expression of brain-derived neurotrophic factor (BDNF) in the hippocampus and the medial prefrontal cortex (mPFC). METHODS: Timed pregnant Wistar rats were delivered on gestation day 14. On postnatal day (PND) 2, litter was assigned to MS or typical animal facility rearing groups. MS was taken place for 3 hours per day for 2 weeks from PND 2 and only male pups were used for the subsequent experiment. On 7 weeks old, the IS session corresponding to trauma was performed, and then 2 weeks after the IS, the avoidance/escape task trials were done. Detailed experimental procedures were described as previously (Sawamura et al, 2004). RESULTS: MS significantly worsened hypoactive behaviors counted as the number of moving to other chamber in the 5 minutes adaptation period ($p<0.05$) and LH behaviors counted as the number of failure to escape ($p<0.01$) and time to successful escape ($p<0.05$) in the avoidance/escape trials. But MS didn't affect to their hyperarousal behaviors. Levels of hippocampal BDNF mRNA didn't decreased by MS, but levels of mPFC BDNF mRNA decreased, especially BDNF exon IV

significantly. CONCLUSIONS: MS worsened not PTSD but LH behaviors in our PTSD model after 2 weeks since the IS, and decreased levels of BDNF mRNA not in the hippocampus but in the mPFC. REFERENCES: Sawamura et al. (2004) Effect of paroxetine on a model of posttraumatic stress disorder in rats. Neuroscience Letters, 357: 37-40.

Disclosures: M. Tanichi: None. **H. Toda:** None. **S. Enomoto:** None. **K. Shimizu:** None. **M. Nagamine:** None. **M. Uenoyama:** None. **M. Nibuya:** None. **A. Yoshino:** None.

Poster

540. Stress: Neural Circuits

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Topic: F.04. Stress and the Brain

Support: NIH grant R01 MH049698

Title: Role of infralimbic cortex in stress responding and extinction memory

Authors: *A. FRANCO-VILLANUEVA[1], R. L. MORANO[2], B. L. SMITH[2], P. MAHBOD[2], J. R. SCHEIMANN[2], B. A. PACKARD[2], B. MYERS[2], J. P. HERMAN[2];
[1]Psychiatry & Behavioral Neurosci., [2]Dept. of Psychiatry and Behavioral Neurosci., Univ. of Cincinnati, Cincinnati, OH

Abstract: The medial prefrontal cortex (mPFC) is critical for emotional control and executive processing in adverse situations. Specifically, individuals suffering from fear or anxiety disorders present PFC hypofunction. In rodents, the mPFC is functionally segregated. Thus, the prelimbic cortex (PL) mediates fear expression and perseveration as well as normal inhibition of the hypothalamic-pituitary-adrenocortical (HPA) axis stress responses, whereas the infralimbic cortex (IL) promotes suppression of fear. Furthermore, chronic stress increases inhibitory synaptic drive to IL pyramidal cells in rats. We used chemogenetics to activate IL neurons in male Sprague-Dawley rats. After confirming the expression of the excitatory Designer Receptor Exclusively Activated by Designer Drugs (DREADD) and its functionality by immunofluorescence, we investigated the role of IL pyramidal neurons on HPA axis stress reactivity and in fear behavior. Chemogenetic activation of IL projection neurons does not affect corticosterone response to acute stress, but does have a lasting effect on fear conditioning one week later. A second IL activation during the extinction retrieval session appears to enhance extinction recall. However, rats expressing the DREADD showed decreased fear behavior on the conditioning day, and thus low levels of freezing during the extinction retrieval could be due to

impaired conditioning. These results suggest that IL engagement in conjunction with stress alters subsequent emotional responses in a glucocorticoid-independent manner.

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Poster

540. Stress: Neural Circuits

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Topic: F.04. Stress and the Brain

Support: NIMH R00 MH097822

R01 MH109685

Hartwell Foundation

International Mental Health Research Organization

Title: Ketamine increases prefrontal dendritic spine formation *In vivo* following chronic stress

Authors: ***M. H. MURDOCK**[1], Y. MENG[1], L. NELLISSEN[1], R. N. MODA[1,2], J. WITZTUM[1,3], C. LISTON[1,2];
[1]Brain and Mind Res. Inst., [2]Neurosci., [3]Physiology, Biophysics and Systems Biol., Weill Cornell Med. Col., New York, NY

Abstract: How ketamine exerts its fast-acting antidepressant effects in the living brain is incompletely understood. Previous work has shown that ketamine, an N-methyl-D-aspartic acid receptor antagonist, increases post-synaptic dendritic spine density in the rat prefrontal cortex. Spines are highly dynamic dendritic protrusions that undergo a constant remodeling process that is shaped by experience. How ketamine affects prefrontal cortical dendritic spine remodeling in the living brain is unknown. Here, we used two-photon microscopy to study the formation and elimination rates of dendritic spines in the frontal association and medial prefrontal cortex of mice expressing yellow fluorescent protein in a subset of layer V pyramidal cells. We used cranial windows or chronically-implanted microprisms to image the same dendritic arbors before and after 21 days of exposure to the murine stress hormone corticosterone. Chronic corticosterone exposure recapitulates an important element of chronic stress which may contribute to depression and other stress-related psychiatric illnesses. We determined that

chronic corticosterone exposure increased spine elimination rates and decreased spine formation rates compared to untreated mice. A single low dose of ketamine (10 mg/kg, intraperitoneal) increased 24-hour spine formation rates following chronic corticosterone exposure. Moreover, a fraction of these newly-formed spines appeared within a distance of 2 micrometers of spines eliminated during corticosterone treatment. These data suggest that ketamine's antidepressant effects are in part due to increased spine formation rates and the recovery of spines lost during chronic stress.

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Poster

540. Stress: Neural Circuits

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Topic: F.04. Stress and the Brain

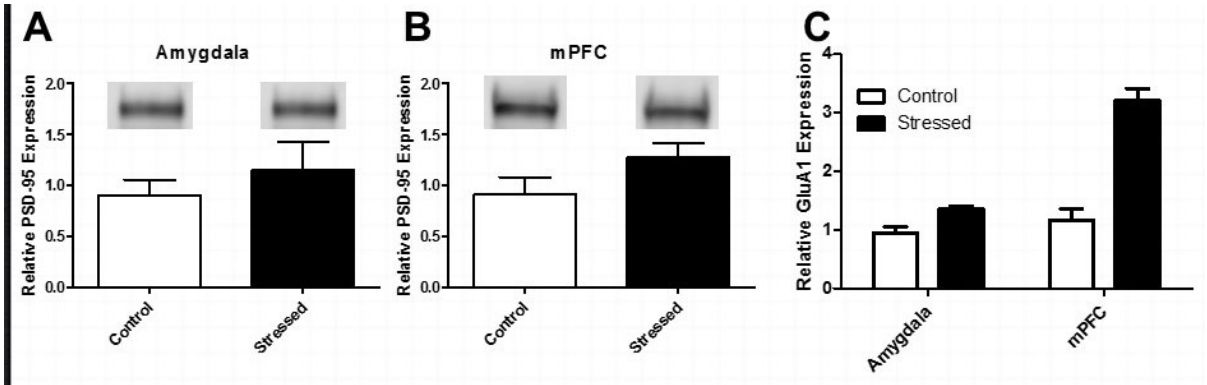
Support: Baylor University

Title: Acute stress increases GluA1 expression in mPFC, but not the amygdala, in adult rats

Authors: ***N. B. KEELE**, M. L. MCREYNOLDS, L. C. ORNELAS;
Baylor Univ. Dept. of Psychology and Neurosci., Waco, TX

Abstract: Environmental stressors are significant antecedents to mood and anxiety disorders. Stress alters both the structure and function of key brain areas involved in emotion, including the medial prefrontal cortex (mPFC) and the amygdala. The amygdala is a critical brain area mediating learned fear/anxiety, and in general, the mPFC acts in a top-down manner to regulate amygdala activity. It is thought that exposure to stress alters amygdala - mPFC circuitry to predispose to stress-related disorders. However, the effects of stress exposure depend on the timing and duration of the stress, as well as the type of stressor, and the brain area under investigation. For example, stress reportedly increases amygdala volume, leading to exaggerated fear behavior. However, stress also causes dendritic atrophy and impairs function of the mPFC. In this project we seek to test the hypothesis that stress affects the synaptic organization differently in the amygdala than the mPFC, and these effects manifest as different anatomical and behavior responses to stress. In control experiments, adult rats with no history of early life stress were subjected to restraint stress (3 days, 1 hr/day). We used standard immunoblotting techniques to determine stress-related changes in PSD-95 and AMPA glutamate receptor 1 (GluA1) expression. Pilot results show that stress did not change PSD-95 expression in either the

mPFC or the amygdala. However GluA1 was increased in the mPFC but not in the amygdala. Increased GluA1 expression may indicate enhanced neural plasticity in the mPFC necessary to elicit adaptive responses to stress. A better understanding of the effects of stress on mPFC-amygdala circuitry may yield important new information regarding the neurobiological underpinnings of pathalogical anxiety.



Stress increases GluA1 expression in the mPFC but not the amygdala. 24 hrs after restraint stress (3 d, 2 hr/d), tissue samples were collected from the amygdala and the medial prefrontal cortex (mPFC) and used for immunoblotting. Postsynaptic density protein of 95kDa (PSD-95) expression was unchanged by stress (filled bars) in both the amygdala (A) and the mPFC (B). However, in these same samples (C), stress caused an increase in the expression of glutamate AMPA receptor subunit 1 (GluA1) in the mPFC (right) but not in the amygdala (left).

Disclosures: **N.B. Keele:** None. **M.L. McReynolds:** None. **L.C. Ornelas:** None.

Poster

540. Stress: Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 540.17/GGG22

Topic: F.04. Stress and the Brain

Support: NSF IOS1456706

Title: Adrenalectomy ablates acute stress-induced Per1 mRNA in the paraventricular nucleus of the hypothalamus, but only attenuates stress-induced Per1 mRNA in the prefrontal cortex of male and female rats

Authors: *L. E. CHUN, J. CHRISTENSEN, E. R. WOODRUFF, S. J. MORTON, L. R. HINDS, R. L. SPENCER;
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Abstract: Properly entrained circadian rhythms optimize survival of an organism. Underlying these circadian rhythms are oscillating molecular clocks, which consists of core clock genes (*Bmal1, Per1, Per2*) whose expression is self-regulating and approximates a 24-hour period. Knockdown of clock genes in rodents leads to aberrant behavior. Thus, optimal health depends on the integrity of the molecular clock, which exists in the body's master clock, the suprachiasmatic nucleus of the hypothalamus (SCN), as well as extra-SCN brain and peripheral tissue. The SCN has few direct efferent projections. Glucocorticoids (CORT) are an ideal candidate for how the SCN entrains extra-SCN molecular clocks. CORT is secreted in a diurnal rhythm, glucocorticoid receptors are ubiquitously expressed (except in the SCN), and *Per1* has a functional glucocorticoid response element. In male rodents, administration of CORT or acute stress increases *Per1* mRNA in peripheral tissue and the paraventricular nucleus of the hypothalamus (PVN). If a diurnal peak in CORT is an entrainment factor for extra-SCN clocks, then untimely, stress-induced surges in CORT can compromise the integrity of the molecular clock. Sex differences exist in stress responsivity and prevalence of mood disorders associated with stress and disruptions in circadian rhythms. Thus, the objective of the present study was to examine whether 30 minutes of acute restraint stress can induce clock gene (*Per1, Per2, Bmal1*) and *C-fos* mRNA (in situ hybridization) in male and female Sprague Dawley rats, and how CORT and estradiol modulates these processes. Rats were housed on a 12:12 h light:dark cycle and received adrenalectomy (ADX) or SHAM surgeries. Female rats were ovariectomized and received estradiol benzoate (10 μg/kg, SC once every 4 days) or vehicle injections. Acute restraint stress rapidly and selectively induced *Per1* mRNA in the PVN and prefrontal cortex (PFC). In male rats this stress-induction was entirely dependent on CORT in the PVN, as ADX prevented stress-induced *Per1* mRNA. However, ADX only attenuated stress-induced *Per1* mRNA in the PFC of male and female rats. Estradiol replacement had no overall effect on *Per1* mRNA in the PFC, but it attenuated stress-induced *C-fos* mRNA in the PFC. These results suggest that regulation of Per1 expression may be the gateway by which stress affects the molecular clock. In addition the results indicate that there are regional differences in the extent to which stress-induced *Per1* mRNA depends on endogenous CORT. Future data analysis will examine *Per2* and *Bmal1* mRNA in the PFC, which may be more affected by estradiol manipulations.

Disclosures: L.E. Chun: None. **J. Christensen:** None. **E.R. Woodruff:** None. **S.J. Morton:** None. **L.R. Hinds:** None. **R.L. Spencer:** None.

Poster

540. Stress: Neural Circuits

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NIH T32GM007739 to the Weill Cornell/Rockefeller/Sloan Kettering Tri-Institutional MD/PhD Program

Title: Effects of chronic social defeat stress on functional connectivity using awake rodent resting state fMRI

Authors: *R. N. FETCHO[1], Y. MENG[2], B. S. HALL[2], T. N. HUYNH[2], C. LISTON[2]; [2]Brain and Mind Res. Inst., [1]Weill Cornell Med. Col., New York, NY

Abstract: The development of resting state functional magnetic resonance imaging (rsfMRI) has provided a powerful tool for noninvasive investigation of circuit mechanisms underlying psychopathology. In humans, rsfMRI has identified a large number of abnormal functional properties in patients diagnosed with stress-related psychiatric disorders. However, interpretation of these results is difficult due to the subjective and heterogeneous nature of psychiatric diagnoses; the diversity of uncontrolled environmental factors impacting disease state; and the purely observational nature of human studies. Recently, rsfMRI has expanded into the realm of rodent research. The discovery of consistent functional networks in rodents, similar to humans, implicates rsfMRI as a promising cross-species technique. Rodent rsfMRI provides an unprecedented opportunity for investigating and manipulating pathological features of functional networks using tools that can be deployed in parallel human studies.
Modeling psychiatric disease in rodents is a difficult task; however, the ability to carefully control the induction of disease-like phenotypes via environmental factors is a major advantage of rodent models. Chronic social defeat stress (CSDS) induces a wide range of “depressive-like” behaviors including anhedonia, anxiety and social dysfunction. Through repeated exposure to and defeat by an aggressive partner, this protocol provides a chronic, naturalistic social stress. CSDS also leads to a variable stress response, with “susceptible” mice showing abnormal

behavioral phenotypes while “resilient” mice do not differ behaviorally from a non-stressed control group. This variability in stress response is interesting in the context of human psychiatric illness, in which an environmental stressor may prime one individual for disease while leaving another unscathed.
Utilizing awake mouse rsfMRI, this work aims to identify changes in functional connectivity associated with stress susceptibility and resilience. Using a within-subject design, mice first undergo rsfMRI to establish baseline non-stressed functional connectivity properties. Mice then enter the CSDS protocol, followed by a second rsfMRI assessment. By comparing functional connectivity in “susceptible” and “resilient” groups, we identify network features important in predicting stress susceptibility as well as network changes that occur in response to chronic stress that correlate with susceptible or resilient behaviors. These findings will facilitate efforts to identify predictors of stress resilience and stress-related pathology identified via rsfMRI in human clinical populations.

Disclosures: **R.N. Fetcho:** None. **Y. Meng:** None. **B.S. Hall:** None. **T.N. Huynh:** None. **C. Liston:** None.

Poster

540. Stress: Neural Circuits

Location: Halls B-H

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Program#/Poster#: 540.19/GGG24

Topic: F.04. Stress and the Brain

Support: NIH R01-MH095972

Title: Prolonged elevations in corticosterone induces regressive and enduring dendritic spine alterations in the medial prefrontal cortex

Authors: ***R. M. ANDERSON**, S. B. JOHNSON, R. GLANZ, S. A. ROMIG-MARTIN, J. J. RADLEY;
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Abstract: The stress-responsive HPA axis plays a central role in promoting adaptations acutely, whereas adverse effects on physiology and behavior following chronic challenges may result from over-activity of this system. Elevations in adrenocortical hormones, the end-products of HPA activation, play roles in adaptive and maladaptive processes by targeting cognate receptors throughout limbic cortical networks to alter synaptic functioning. Work from our laboratory and others have shown that chronic stress leads to functionally- relevant regressive alterations in dendritic spine shape and number in pyramidal neurons in the medial prefrontal cortex (mPFC),

thus implicating a glucocorticoid-dependency for these effects. Here we examined the nature and temporal characteristics of prolonged glucocorticoid exposure on dendritic spine modifications in the mPFC. Rats were implanted with subcutaneous pellets of corticosterone (B) to provide for the continuous release over a 7-, 14-, 21-day period, and 21 days of exposure + 21-day washout. Plasma B measurements in clamped rats using radioimmunoassay revealed steroid levels that approximated the circadian mean (150 ng/ml) with respect to sham-pellet control rats. Animals were perfused and pyramidal neurons throughout all layers of prelimbic region (PL) of mPFC were selected for intracellular fluorescent dye-filling, followed by high-resolution 3D imaging and analysis of dendritic arborization and spine morphometry. Whereas no effects were observed following 7-day exposure, 14 days of B exposure decreased apical dendritic length (by 23%; $p < 0.05$) and spine density (by 13% for each; $p < 0.05$ for each). Thin spine subtypes showed the greatest degree of attrition throughout apical dendrites (by 12%, $p < 0.05$ for each). Follow-up population analyses of spine characteristics in rats receiving B for 2 weeks revealed decreases in spine volume across subtypes. After 21 days of B exposure, similar effects were observed as with 14-day treatment, although the effects of spine attrition extended to larger volume subtypes. Moreover, the chronicity of glucocorticoid-induced dendritic spine loss in PL was highlighted by the failure of these indices to normalize following a 21-day washout period. These results suggest that prolonged alterations in adrenocortical activity may be sufficient to induce enduring regressive structural alterations in mPFC, and may be relevant for understanding mechanisms of mPFC compromise in a variety of contexts whereby glucocorticoids become dysregulated, such as aging, chronic stress, and Cushings disease.

Disclosures: R.M. Anderson: None. **S.B. Johnson:** None. **R. Glanz:** None. **S.A. Romig-Martin:** None. **J.J. Radley:** None.

Poster

540. Stress: Neural Circuits

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Topic: F.04. Stress and the Brain

Support: NIMH R00 MH097822

R01 MH109685

Hartwell Foundation

International Mental Health Research Organization

National Science Foundation Graduate Research Fellowship Grant No. 1257284

Title: Network analysis of frontal cortical microcircuit dynamics after chronic stress hormone exposure and ketamine treatment

Authors: *R. N. MODA[1,3], R. N. FETCHO[1,3], J. WITZTUM[2,3], M. H. MURDOCK[3], Y. MENG[3], C. LISTON[1,3];
[1]Neurosci., [2]Physiology, Biophysics and Systems Biol., Weill Cornell Med. Col., New York, NY; [3]Brain and Mind Res. Inst., New York, NY

Abstract: Chronic stress exposure has been shown to alter neuronal morphology within the prefrontal cortex (PFC), inducing changes such as dendritic retraction and spine loss. Additionally, clinical studies have shown that subanesthetic doses of ketamine, an NMDA antagonist, can act as a fast-acting antidepressant in treatment-resistant depressed patients. These effects may be mediated by a ketamine-induced increase in postsynaptic dendritic spine density in PFC pyramidal cells. How changes in synapse number and dendritic morphology affect PFC microcircuit function, however, has yet to be established.
In an effort to address this question, we investigated neural network dynamics in dorsal PFC microcircuits using two-photon calcium imaging. Adult mice were intrancranially injected in the prefrontal cortex with a pan-neuronal GCaMP-expressing AAV virus (driven by the hSyn promoter), and a borosilicate glass cranial window was chronically implanted in the skull for visualization of GCaMP-expressing cells. PFC microcircuit activity was quantified by two-photon calcium imaging before and after a chronic, 21-day exposure to high levels of corticosterone, the principal murine stress hormone, via corticosterone tablets that were implanted subcutaneously. The mice were then injected with a subanesthetic intraperitoneal dose of ketamine (10 mg/kg IP), and repeat imaging was performed 24 hours later from the same neural populations. PFC microcircuit activity and network properties were analyzed, and results suggest that chronic corticosterone exposure suppresses neuronal activity and alters functional connectivity in the PFC. Following a single injection of ketamine, these effects are partially rescued, with an increase in cell activity that is especially pronounced in highly connected, "hub-like" neurons. Further, analysis of graph theory metrics of PFC network connectivity suggests that ketamine also partially rescues corticosterone-induced deficits in network connectivity measures.

Disclosures: **R.N. Moda:** None. **R.N. Fetcho:** None. **J. Witztum:** None. **M.H. Murdock:** None. **Y. Meng:** None. **C. Liston:** None.

Poster

540. Stress: Neural Circuits

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Program#/Poster#: 540.21/GGG26

Topic: F.04. Stress and the Brain

Support: R01 MH050479 (SFM)

R21 MH106817 (MVB)

T32 HD7289-30 (SDD)

Title: Circuit-specific dendritic spine changes after controllable or uncontrollable stress in medial prefrontal cortex of male and female rats.

Authors: *T. GRUENE[1], M. V. BARATTA[3], S. D. DOLZANI[4], L. E. CHUN[3], S. F. MAIER[3], R. M. SHANSKY[2];
[2]Dept. of Psychology, [1]Northeastern Univ., Boston, MA; [3]Dept. of Psychology and Neurosci., [4]Dept. of Psychology and Neuroscience; Inst. for Behavioral Genet., Univ. of Colorado Boulder, Boulder, CO

Abstract: The stressor controllability paradigm has been extensively used to demonstrate that control over stress leads to adaptive outcomes and blunts the effects of the stressor. In this paradigm rats are either exposed to escapable tailshock (ES), yoked inescapable tailshock (IS), or no stress. While IS leads to a variety of behavioral effects such as increased anxiety and social avoidance, ES animals are protected from these effects. Moreover, ES "immunizes" animals from the negative effects of future stressors. The protective and long term "immunizing" effects of behavioral control depend on the activation of prelimbic cortex (PL) neurons that project to the dorsal raphe nucleus (DRN). The foregoing suggests that DRN-projecting PL neurons undergo stable changes following ES, thus it is possible that ES leads to dendritic spine changes that support these stress-buffering effects. How ES and IS impacts dendritic morphology in the PL-to-DRN pathway is unknown. Here we investigate the possibility that ES induces distinct dendritic spine changes in a circuit-specific manner in both male and female rats. DRN-projecting PL neurons were labeled via fluorescent retrobead injections into the DRN. Then, rats were exposed to either ES, IS or no stress and were sacrificed 24 hours later for morphology analysis. Microinjections of fluorescent dye were targeted to retrobead labeled and unlabeled neighboring neurons. Dendritic segments were imaged in 3D using a confocal microscope and automated spine analysis was performed. In addition to overall spine density, spine type (mushroom or thin) density as well as spine head diameters were analyzed. By comparing morphology of DRN- to non DRN-projecting PL neurons, we are able to perform within-subject analyses to identify circuit-specific spine changes as well as investigate group differences in

spine density of labeled and unlabeled PL neurons. Additionally, we report analyses comparing dendritic spine effects in males and females to uncover possible sex-differences.

Disclosures: **T. Gruene:** None. **M.V. Baratta:** None. **S.D. Dolzani:** None. **L.E. Chun:** None. **S.F. Maier:** None. **R.M. Shansky:** None.

Poster

540. Stress: Neural Circuits

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Program#/Poster#: 540.22/HHH1

Topic: F.04. Stress and the Brain

Support: DARPA and Army Research Office W911NF1010093

Title: Investigating the role of the paraventricular thalamic nucleus in regulating the stress response: potential molecular mechanisms

Authors: B. CORBETT[1], S. LUZ[1], *S. BHATNAGAR[2];
[1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Dept Anesthesiol., Univ. Pennsylvania, Children's Hosp Philadelphia, Philadelphia, PA

Abstract: In the field of stress neurobiology, habituation is defined as a decreasing and learned response to a familiar stressor over time. Disrupted habituation is a signature of post-traumatic stress disorder (PTSD), causing devastating effects for those afflicted. Understanding the molecular and neural substrates underlying habituation may allow for improved therapies for PTSD patients. In rats, we model habituation using the repeated restraint paradigm. Repeated exposure to this moderately intense stressor increases the expression of immediate early genes in certain brain regions, induces the production of stress-related hormones, and elicits struggle behavior. All of these responses are highest on day 1 of restraint and attenuate over time. We have previously identified the posterior paraventricular thalamic nucleus (pPVT) as a crucial brain region that regulates habituation. However, the underlying molecular mechanisms and specific neural connections between the pPVT and other brain regions that mediate habituation are unknown. Using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), we investigating the role of the pPVT in regulating the stress response. We demonstrated that chemogenetic inhibition of the pPVT increased adrenocorticotropic hormone production on day 1 and day 5 in rats receiving injections of clozapine-N-oxide (CNO), the synthetic ligand for DREADDs, compared to controls. Further, chemogenetic inhibition of the pPVT attenuated corticosterone habituation as increased corticosterone levels were observed on day 5, but not day 1, in CNO-treated rats. We are currently investigating whether a subset of pPVT neurons that

project to the medial prefrontal cortex (mPFC), another brain region that negatively regulates the stress response, mediates habituation. We hypothesize that pPVT neurons, in particular those that project to the mPFC, play a critical role in stress habitation. Additionally, we investigated a potential mechanisms of neuronal habituation within the pPVT by assessing the expression of activity-regulated cytoskeleton-associated protein (Arc) in naïve rats. Arc is an immediate early gene that reduces excitatory synapse number. We found that Arc expression was increased in the pPVT on day 1, but not day 5 of restraint. We hypothesize that Arc-mediated restrictions in excitatory synapse number are a critical mechanism underlying habituation of the stress response. Our findings offer new insight into the role of the pPVT in mediating the stress response and are among the first to provide a possible molecular mechanism of stress habituation.

Disclosures: B. Corbett: None. **S. Luz:** None. **S. Bhatnagar:** None.

Poster

540. Stress: Neural Circuits

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Topic: F.04. Stress and the Brain

Support: Danish Technical University

Danish Ministry of Food, Agriculture and Fisheries

University of Copenhagen

Title: Effects of acute and chronic stress on telencephalic neurochemistry and gene expression in rainbow trout (Oncorhynchus mykiss)

Authors: *M. MOLTESEN[1], D. C. LAURSEN[2], P.-O. THÖRNQVIST[3], M. Å. ANDERSSON[4], T. DABELSTEEN[1], S. WINBERG[3], E. HÖGLUND[5];
[1]Univ. of Copenhagen, Copenhagen, Denmark; [2]Danish Tech. Univ., Hirtshals, Denmark; [3]Uppsala Univ., Uppsala, Sweden; [4]Lund Univ., Lund, Sweden; [5]Norsk institut for vannforskning, Grimstad, Norway

Abstract: By filtering relevant sensory inputs and initiating stress responses, the brain is an essential organ in stress coping and adaptation. However, exposure to chronic or repeated stress can lead to allostatic overload, where neuroendocrinal and behavioral reactions to stress become maladaptive. This work examines forebrain mechanisms involved in allostatic processes in teleost fishes. Plasma cortisol, forebrain serotonergic (5-HTergic) neurochemistry and mRNA

levels of corticotropin-releasing factor (CRF), CRF binding protein (CRFBP), CRF receptors (CRFR1 and CRFR2), mineralcorticoid receptor (MR), glucocorticoid receptors (GR1 and GR2), and 5-HT $_{1A}$ receptors (5-HT$_{1Aa}$ R and 5-HT$_{1Ab}$ R) were investigated at 1 h before and 0, 1 and 4 h after acute stress, in two groups of rainbow trout held in densities of 25 and 140 kg m^{-3} for 27 days. Generally, being held at 140 kg m^{-3} resulted in a less pronounced cortisol response. This effect was also reflected in lower forebrain 5-HTergic turnover, but not in mRNA levels in any of the investigated genes. This lends further support to allostatic load as a situation where fish cannot mount a proper cortisol in response to an acute stressor, and suggests that changes in forebrain 5-HT metabolism is involved in allostatic processes in fish. Independent of rearing densities, mRNA levels of 5-HT $_{1Aa}$ R and MR were down regulated 4 h post stress compared to values 1 h post stress, suggesting that these receptors are under feedback control and take part in the down regulation of the hypothalamic-pituitary-interrenal (HPI) axis after an acute stressor.

Disclosures: **M. Moltesen:** None. **D.C. Laursen:** None. **P. Thörnqvist:** None. **M.Å. Andersson:** None. **T. Dabelsteen:** None. **S. Winberg:** None. **E. Höglund:** None.

Poster

541. Drug Addiction: Learning and Memory

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 541.01/HHH3

Topic: G.08. Drugs of Abuse and Addiction

Support: Hanyang University HY-2016

Title: Neurobiological findings related to Internet use disorders

Authors: ***S. ROH**[1], B. PARK[2], D. HAN[3];
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Abstract: In the last ten years, numerous neurobiological studies have been conducted on Internet addiction or Internet use disorder. Various neurobiological research methods—such as magnetic resonance imaging; nuclear imaging modalities, including positron emission tomography and single photon emission computed tomography; molecular genetics; and neurophysiologic methods—have made it possible to discover structural or functional impairments in the brains of individuals with Internet use disorder. Specifically, Internet use disorder is associated with structural or functional impairment in the orbitofrontal cortex, dorsolateral prefrontal cortex, anterior cingulate cortex, and posterior cingulate cortex. These

regions are associated with the processing of reward, motivation, memory, and cognitive control. Early neurobiological research results in this area indicated that Internet use disorder shares many similarities with substance use disorders, including, to a certain extent, a shared pathophysiology. However, recent studies suggest that differences in biological and psychological markers exist between Internet use disorder and substance use disorders. Further research is required for a better understanding of the pathophysiology of Internet use disorders.

Disclosures: **S. Roh:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; Hanyang University. **B. Park:** None. **D. Han:** None.

Poster

541. Drug Addiction: Learning and Memory

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 541.02/HHH4

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIAA R37AA011852

Title: Reducing alcohol cue reactivity in rats using retrieval+extinction

Authors: *R. U. COFRESI[1], S. M. LEWIS[1], K. M. TUITE[1], I. S. PARK[1], N. CHAUDHRI[2], H. J. LEE[1], M. H. MONFILS[1], R. A. GONZALES[1];
[1]The Univ. of Texas At Austin, Austin, TX; [2]Concordia Univ., Montréal, QC, Canada

Abstract: Conditioned responses to alcohol-associated cues can hinder an indvidual's attempts to abstain from or moderate their drinking. Cue extinction protocols can reduce reactivity to alcohol cues, but their efficacy is limited. Here, we evaluated the potential of memory retrieval-enhanced cue extinction to reduce return of extinguished responses to alcohol-predictive cues relative to return of responses observed following standard cue extinction. To do this, we used our recently developed model of alcohol cue learning (presented by Cofresí et al, SfN 2015): a discrete trial procedure in which time-limited access to unsweetened alcohol (via retractable sipper) is contingent upon a visual cue (houselight illumination). We adapted the extinction phase in our model such that every day rats experienced context exposure (NORET) or isolated cue retrieval (RET) and following 1hr in homecage, a standard cue extinction session. Total exposure to cue and context within and across the 14 consecutive days was equated. RET and NORET groups were also matched on ingested ethanol doses across conditioning as well as levels of visual cue-conditioned anticipatory approach to the site of alcohol access and contact

with the alcohol access device (sipper). We tested for early spontaneous recovery of extinguished responses by giving a 4-trial extinction probe 48 hr after the last extinction session (SR test). We tested for reinstatement of extinguished responses using a second memory probe during which ethanol odor was present (RT test). We also evaluated response reacquisition rate by reintroducing cue-contingent alcohol access. Rats exhibited similarly low response likelihoods over the final 4 extinction trials, but differed over SR and RT test trials depending on NORET v. RET treatment. Approach and contact by NORET rats (n=14) decayed slowly over trials from initially high levels in both tests. In contrast, responses by RET rats (n=13) were initially low and either did not change or increased modestly before rapidly decaying across trials in both tests. In keeping with a protective effect of RET treatment against response return, RET rats (n=6) were also slower to reacquire responses to the cue relative to NORET rats (n=7). Our findings strongly suggest that isolated cue memory retrieval prior to cue extinction may enhance the efficacy of cue exposure therapy to reduce alcohol cue reactivity.

Disclosures: **R.U. Cofresi:** None. **S.M. Lewis:** None. **K.M. Tuite:** None. **I.S. Park:** None. **N. Chaudhri:** None. **H.J. Lee:** None. **M.H. Monfils:** None. **R.A. Gonzales:** None.

Poster

541. Drug Addiction: Learning and Memory

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 541.03/HHH5

Topic: G.08. Drugs of Abuse and Addiction

Support: James S. McDonnell Foundation

Kavli Institute for Brain and Mind

Title: A novel behavioral paradigm for assessing the role of behavioral pattern separation in ethanol relapse

Authors: ***P.-K. HUANG**[1], E. H.-J. WANG[1], E. MEJIA[2], L. K. QUINN[1], F. H. GAGE[2], A. A. CHIBA[1];
[1]Univ. of California San Diego, La Jolla, CA; [2]Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: A major obstacle facing the problem of drug addiction is the resilient nature of drug relapse during protracted abstinence. Overcoming this obstacle requires a greater understanding of how certain drug-induced changes in the brain can produce an aberrant chronic relapsing state that is observed in drug addiction. Recent research examining the behavioral role of neurogenesis in the hippocampus enables new avenues for exploration that may provide important insights to

understanding the progression of drug relapse in addiction. The dentate gyrus and neurogenesis are thought to be important for discriminating between similarly encoded contexts, often referred to and demonstrated experimentally as behavioral pattern separation. Additionally, chronic consumption of addictive drugs across different drug classes has been widely shown to impair neurogenesis. However, the role that behavioral pattern separation plays in drug addiction has yet to be explored. This study tests the hypothesis that a gradual decline in neurogenesis from repeated drug consumption leads to impairments in the ability to discriminate between drug-paired contexts and other similar non-drug-paired contexts. With continual drug use, drug craving and relapse may be experienced in dissimilar contexts. Thus, a propensity for relapse may arise from the loss of specificity of the encoded associations between the affective properties of the drug and the contexts in which the drug was taken. This phenomenon of context overgeneralization, presumably due to the loss of ability to discriminate fine differences between contexts may reside at the heart of the observation of drug cravings and relapse gradually becoming more prevalent with the progression from recreational drug use to addiction. To test this hypothesis, this study implements a new behavioral paradigm to simultaneously assess discrimination of similar visual contextual memories (behavioral pattern separation) and preference for contexts associated with drug and natural rewards (relapse-like behavior) in chronic ethanol-drinking rats and non-ethanol controls.
Acknowledge: James S. McDonnell Foundation; Kavli Institute for Brain and Mind

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Poster

541. Drug Addiction: Learning and Memory

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Training Grant T32 DA024635

Title: Neural mechanisms of memory system bias following withdrawal from cocaine.

Authors: *E. HARVEY[1], C. S. AHN[2], N. ANGELILIIS[2], P. J. KENNEDY[2];
[1]Psychology, [2]Univ. of California Los Angeles, Los Angeles, CA

Abstract: Research in the field of learning and memory suggests that the brain is composed of multiple memory systems. Among these systems is the hippocampus (HC), which encodes information flexibly and supports behaviors that can be rapidly updated. Another system is the

dorsolateral striatum (DLS), which encodes information inflexibly and supports behaviors that are “hard-wired” (habits). During new learning, humans with a history of addictive drug use will show a bias towards learning and memory processes that are dependent upon the dorsolateral striatum (DLS), and deficits in hippocampal-dependent memory processing have been shown to predict poor treatment outcomes in stimulant users. Here we investigated the effects of prior cocaine exposure on performance in a dual solution cross-maze task that tests the use of flexible, HC-dependent versus inflexible, DLS-dependent learning strategies. We found that following 3 weeks of withdrawal from chronic cocaine, rats demonstrated a bias towards the use of a DLS-dependent learning strategy whereas drug naïve controls demonstrated learning dependent on the HC. This effect was reversed by peripheral treatment with a kappa opioid antagonist (JDTic) throughout withdrawal. Epigenetic mechanisms contribute to both maladaptive neuronal plasticity associated with drug exposure, and brain plasticity that is critical for memory formation. We further report that withdrawal from chronic cocaine is associated with an increase in global levels of permissive histone modifications within the DLS, and a reduction within the HC. These data suggest a molecular switch that may contribute to persistent changes in DLS and HC plasticity following repeated exposure to cocaine. Follow-up experiments are underway to investigate transcriptional adaptations mediating drug-induced memory system and behavioral bias and the effects of kappa opioid antagonism on these processes.

Disclosures: **E. Harvey:** None. **C.S. Ahn:** None. **N. Angeliliis:** None. **P.J. Kennedy:** None.

Poster

541. Drug Addiction: Learning and Memory

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 541.05/HHH7

Topic: G.08. Drugs of Abuse and Addiction

Title: Extinction of morphine place-preference and neuroplasticity transcript profile of the ventral striatum

Authors: *M. E. LLORET, F. MARTINEZ;
Univ. of Puerto Rico Med. Sci. Campus, San Juan, Puerto Rico

Abstract: Drug addiction has been associated with deficits in neural circuits that regulate extinction of addictive behaviors. Current available data correlate addiction to neuroplasticity changes in the reward circuit, however little is known about the cellular and molecular mechanisms for extinction learning. We used conditioned place preference (CPP) to identify differential expression of plasticity genes in the ventral striatum/nucleus accumbens (VS/Nac) of extinction-trained animals. Rats expressing morphine (5mg/kg) CPP were divided into 2 groups

receiving either forced or sham extinction training during 4 consecutive days. After the extinction test day the VS/Nac was isolated and differentially expressed genes were examined using RT² Profiler™ PCR array kit for plasticity genes. We identified two separate animal's subgroups: those that extinguished CPP (Extinction-CPP) and those still showing CPP (Persistent-CPP). As expected, extinction-untrained animals (Sham-Extinction) retained their morphine place preference. Real time RT-PCR analysis revealed differential gene expression for the extinction of morphine-seeking behavior. Notably, rats the Extinction-CPP group showed upregulation of Bdnf, Gria 4 and Reln transcripts. In the Persistent-CPP group, transcripts for Crem and Rheb were upregulated, while Tnfa was downregulated. On the other hand, Sham-Extinction rats showed a widespread modulation of synaptic plasticity genes including transcript upregulation of Camk2g, Ephb2, Grm8 and Ngfr, as well as mu and delta opioid receptors. Together, our data, allowed us to compare different phenotypes of extintion learning based on differential modulation of gene expression. It could also suggest that alteration of plasticity genes could alter extinction learning and/or vice versa. Future studies at the protein level in the reward system i.e. amygdala, prefrontal cortex and Hippocampus will be evaluated, as well as pharmacological studies to demonstrate the role of key molecular players (i.e. Bdnf and opioid receptors) regulating extinction behaviors.

Disclosures: **M.E. Lloret:** None. **F. Martinez:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: DGAPA-UNAM Grant IN218316 to OPG

DGAPA-UNAM Grant IA207416 to MMD

DGAPA-UNAM Grant IN219516 to AERC

Title: CB1 receptor decreased expression in the nucleus accumbens and prefrontal cortex prevents amphetamine-induced place preference conditioning in rats

Authors: ***D. PEREZ**[1], A. ROMANO-LOPEZ[1], A. RUIZ-CONTRERAS[2], M. MENDEZ-DIAZ[1], O. PROSPERO-GARCIA[1];
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Abstract: An extensive literature suggests drugs of abuse-dependence is a learning process. Memory consolidation, including memory re-consolidation, requires protein synthesis. In this context, we decided to used chloramphenicol (CAP), a drug that inhibits bacterial but also mammalian protein synthesis to prevent amphetamine (amph)-seeking behavior. Twenty Wistar adult rats were subjected to amph (2mg/kg)-induced Conditioned Place Preference (CPP). Rats were divided into 2 groups (n=10). Amph+saline (veh) rats, received amph and were kept in one compartment of the chamber (30min). Once this period finished they received a subcutaneous injection of veh (0.5ml) and returned to their home-cage. Amph+CAP rats, were similarly treated with amph but instead of veh received CAP (150mg/kg). Once CPP was evaluated rats were killed and the prefrontal cortex (PFC) and the nucleus accumbens (NAcc) were isolated and prepared for CB1 receptor Western blot analysis. A vivarium (viv) reared group of rats (n=8) was added as control. Results indicated that rats receiving amph+veh developed CPP while increasing CB1R expression in the NAcc, with no changes in the PFC compared to viv-control. Rats receiving amph+CAP did not develop CPP while reducing CB1R expression in the PFC compared to both viv- and amph+veh-rats. Regarding CB1R in the NAcc, amph+CAP amount was similar to the amount of viv-control, sparing the increase detected in the amph+veh, suggesting CAP prevented the amph-induced CB1R increase. These results further support the notion that drug-dependence is a learning process that requires protein synthesis. In the case of amph-induced CPP includes an increase of CB1R in the NAcc that is prevented by protein synthesis inhibitor CAP.

Disclosures: D. Perez: None. **A. Romano-Lopez:** None. **A. Ruiz-Contreras:** None. **M. Mendez-Diaz:** None. **O. Prospero-Garcia:** None.

Poster

541. Drug Addiction: Learning and Memory

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Topic: G.08. Drugs of Abuse and Addiction

Support: R01 DA038042

Title: 17β-estradiol enhances intrinsic excitability of IL-mPFC neurons through a BDNF-dependent mechanism

Authors: *H. YOUSUF[1], D. MUELLER[2];
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Abstract: Persistence of strong emotional memories can lead to the development of neuropsychiatric disorders such as posttraumatic stress disorder (PTSD) and drug addiction. Treatment of maladaptive memories can occur through extinction learning, which involves the formation of a new inhibitory memory and suppresses fear expression and drug seeking. (Mueller and Quirk, 2008; Quirk et al., 2006). The infralimbic medial prefrontal cortex (IL-mPFC) is critical for extinction of conditioned fear and dug seeking (Milad and Quirk, 2002; Otis et al., 2014). The potent estrogen, 17β-estradiol (E_2), has been shown to increase dendritic spine density and memory function. Behaviorally, E_2 enhances extinction of both conditioned fear and cocaine seeking (Twining et al., 2013; Graham and Milad, 2014). However, the cellular mechanisms by which E_2 enhances extinction remain unclear. Using patch-clamp electrophysiology, we tested whether E_2 alters intrinsic excitability in IL-mPFC neurons as intrinsic excitability promotes synaptic plasticity underlying memory formation. Brain slices were derived from female ovariectomized rats and recordings were obtained from layer V IL-mPFC pyramidal neurons. The minimum amount of current required to elicit a single action potential was used as a depolarizing step throughout the experiments. Bath-application of E_2 significantly increased excitability as compared to vehicle treatment in IL-mPFC neurons. Activity of E_2 is modulated via several signaling molecules, including brain-derived neurotrophic factor (BDNF). Therefore, we tested whether E_2-induced potentiation of intrinsic excitability in the IL-mPFC is mediated via BDNF signaling. Since, BDNF binds to its high-affinity tropomyosin receptor kinase B (TrkB), slices were incubated with a Trk receptor antagonist, K-252a, prior to bath-application of E_2. Patch-clamp recordings from IL-mPFC pyramidal neurons revealed that E_2-induced enhancement of intrinsic excitability was prevented in the presence of K-252a. Thus, E_2 enhances intrinsic excitability in a TrkB-dependent manner. Furthermore, this enhanced excitability may underlie E_2-induced facilitation of extinction of conditioned fear and drug seeking. These data indicate that optimizing E_2 levels and/or augmenting BDNF function could enhance therapeutic interventions to alleviate neuropsychiatric disorders such as PTSD and drug addiction.

Disclosures: **H. Yousuf:** None. **D. Mueller:** None.

Poster

541. Drug Addiction: Learning and Memory

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Topic: G.08. Drugs of Abuse and Addiction

Support: R01 DA038042

Title: Expression of basic fibroblast growth factor (bFGF or FGF2) is regulated by extinction following cocaine self-administrtion

Authors: *M. HAFENBREIDEL[1], C. W. SMIES[1], D. MUELLER[2];
[1]Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Ponce Hlth. Sci. University-School of Med., Ponce, PR

Abstract: Drug addiction is characterized by compulsive drug seeking and chronic relapse, which are maintained by drug-induced maladaptive plasticity. This plasticity is mediated, in part, by the actions of growth factors such as basic fibroblast growth factor (bFGF or FGF2). bFGF expression is increased following stimulant drug use in reward-related brain regions, including the medial prefrontal cortex (mPFC; Fumagalli et al., 2006; Hafenbreidel et al., 2015), and bFGF mediates drug-induced increases in dendritic length (Mueller et al., 2006) as well as amphetamine-induced sensitization (Flores et al., 2000). Moreover, addiction is maintained by cues associated with the drug, and therefore reducing cue reactivity through extinction could reduce relapse rates. We previously found that neutralizing bFGF in the infralimbic region of the mPFC (IL-mPFC) facilitates extinction following cocaine self-administration (Hafenbreidel et al., 2015). However, whether bFGF overexpression is sufficient to prolong extinction and what mechanisms underlie bFGF-induced resistance to extinction is unknown. Therefore, we first determined if bFGF overexpression would delay new learning. Extinction of sucrose self-administration does not alter bFGF expression in IL-mPFC (Hafenbreidel et al., 2015), so we overexpressed bFGF in this region by infusing bFGF prior to extinction. First, rats were trained to lever press for sucrose before undergoing extinction, which consisted of eight 90 min extinction sessions. Next, rats were infused with vehicle or bFGF into IL-mPFC prior to the first four extinction sessions. We found that bFGF-treated rats extinguished at the same rate as vehicle-treated rats, indicating that increasing infralimbic bFGF during extinction was not sufficient to prolong extinction. Thus, exogenous bFGF neither enhances nor impairs extinction of sucrose seeking. In contrast, we previously reported that extinction reverses drug-induced bFGF overexpression in IL-mPFC that occurs following self-administration (Hafenbreidel et al., 2015). Additionally, preliminary analyses suggest that extinction following cocaine self-administration also reduces bFGF protein expression in the nucleus accumbens and dorsal hippocampus, and ongoing investigations are examining the mechanisms underlying these effects. In summary, extinction reduces bFGF expression throughout reward-related circuitry, indicating that targeted reductions in bFGF expression could facilitate addiction treatment.

Disclosures: **M. Hafenbreidel:** None. **C.W. Smies:** None. **D. Mueller:** None.

Poster

541. Drug Addiction: Learning and Memory

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Program#/Poster#: 541.09/HHH11

Topic: G.08. Drugs of Abuse and Addiction

Title: Brain activation patterns associated with cue reactivity and craving in Internet gaming disorder: an fMRI study

Authors: *J.-E. JEONG;
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Abstract: *Background and aims*: Extensive use of the Internet gaming has become widespread and problematic for the last two decades. This study aimed to investigate the brain activation pattern associated with cue reactivity and craving in Internet gaming disorder (IGD). *Methods: Participants* The sample included 23 healthy control (HC) males (age, 30.04 ± 5.17) and 25 males with the Internet gaming disorder (IGD) (age, 30.27 ± 5.27) were selected using IGD criteria which recently included in section III of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). Both of the HC and IGD group had an experience of playing the same kinds of games. *Procedures* Before the fMRI scanning, the Internet gaming video was presented for 1 minute in order to maximize the craving response. The video was made of the highlights from the online videogame matches. The gaming craving was measured, by asking to the extent of urge to play the video game at this moment, using the 10 point visual analog scale after presentation of the video clip. During the fMRI scanning, participants viewed the stimulus that consisted of three sets of picture: gaming, mosaic, and landscapes. These pictures were arranged according to a block design. *Results*: In the results of perceived gaming craving, the participants with IGD recorded higher scores than with HC ($t=-2.0$, $df=47$, $p<.05$). In the fMRI results, the IGD group compared to HC revealed more activation on right dorsolateral prefrontal cortex (DLPFC), left occipital lobe, bilateral caudate nucleus, and right medial prefrontal cortex (MPFC) under gaming pictures (relative to mosaic pictures). On the other hand, brain activity within left ventrolateral prefrontal cortex (VLPFC) and anterior part of left prefrontal cortex was decreased in IGD group compared to HC under gaming pictures (relative to landscapes pictures). *Conclusions*: In this study, we could demonstrate the different cerebral activation between normal Internet game users and people with IGD during watching cue-induced gaming pictures. In the fMRI results of the gaming condition, the IGD group compared with HC showed more activation in DLPFC and left occipital cortex, related to regulation of craving and processing the visual information. In addition, activation in caudate nucleus, which is involved in reward circuit, would be associated with the subjective experience of gaming craving. On the other hand, IGD group compared with HC showed deactivation in VLPFC, which have been reported as the important area of the cognitive reappraisal and emotion regulation.

Disclosures: J. Jeong: None.

Poster

541. Drug Addiction: Learning and Memory

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Title: Involvement of histone deacetylase 3 in extinction of reward-seeking behaviors

Authors: *L. N. HITCHCOCK[1], T. M. NAVIS[1], M. A. WOOD[2], K. M. LATTAL[1]; [1]Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR; [2]Neurobio. & Behavior, Univ. of California-Irvine, Irvine, CA

Abstract: Substance use disorder is a chronic, often relapsing disease that leads to a loss of behavioral inhibition and compulsive drug-seeking. Cues that are paired with acquisition of drug-seeking are thought to influence subsequent extinction (animal model of exposure-based therapy) and relapse-like behavior, both in humans and animals. Our previous work has implicated the dorsal hippocampus for developing and retrieving memories in a drug-seeking context. By inactivating the dorsal hippocampus, we found that extinction was impaired in a contextual learning paradigm (cocaine-induced conditioned place preference). At the molecular level, the epigenetic enzyme histone deacetylase 3 (HDAC3), has been shown to be a negative regulator of cocaine-associated learning and memory. Here, we further investigate this hippocampus-based extinction model and determine whether inhibition of HDAC3 can enhance extinction after cocaine self-administration. Extended extinction did not eliminate contextual renewal or cue-induced reinstatement, but a systemic injection of an HDAC3 inhibitor caused persistent extinction and weakened renewal and cue-induced reinstatement. We examined the generality of these findings by assessing effects of a viral point mutation of HDAC3 in the dorsal hippocampus during acquisition and extinction of responding for natural rewards (food pellets). In this experiment, the HDAC3 point mutant led to faster acquisition and faster extinction learning. Together, these findings support the idea that HDAC3 is involved in acquisition and extinction of reward-seeking behaviors.

Disclosures: **L.N. Hitchcock:** None. **T.M. Navis:** None. **M.A. Wood:** None. **K.M. Lattal:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA034116

NIH Diversity Supplement

Title: Inhibition of hippocampal nonmuscle myosin II disrupts the reconsolidation of methamphetamine-associated memory

Authors: ***S. B. BRIGGS**, E. J. YOUNG, A. M. BLOUIN, G. RUMBAUGH, C. A. MILLER; Neurosci., The Scripps Res. Institute-Fl Campus, Jupiter, FL

Abstract: Persistent, drug-associated memories are an underlying core feature of substance use disorder (SUD). These memories are trigged by numerous and often abstract environmental cues, making them difficult to predict and treat. Dendritic spines, which are thought to contribute to the encoding and storage of memory, undergo actin-dependent changes that are critical to long-term memory. We recently reported that the actin cytoskeleton that supports methamphetamine (METH)-associated memories, but not other amygdala (AMY)- dependent memories, continuously cycles, presenting a new and unique therapeutic target for METH abuse that is both selective and retrieval-independent. Moreover, disrupting actin polymerization with a single, intra-AMY or systemic administration of Blebbistatin (Blebb), a nonmuscle myosin II inhibitor, abolishes METH seeking, with a concomitant decrease in AMY spine density, providing a potential therapeutic target. Presently, we sought to understand if the mechanism by which Blebb disrupts METH-associated memory is unique to the AMY. To test this, male mice received Blebb infusions into other regions of the circuit supporting drug-associated memories, the prefrontal (PFC), nucleus accumbens (NAc) or the hippocampus (Hipp), immediately before METH conditioned place preference testing. Contrary to our AMY results, Bebb injections into these regions did not result in an immediate disruption of drug seeking, suggesting that the actin cytoskeleton in the PFC, NAc and Hipp is stable after METH exposure. Furthermore, unlike in the AMY, the METH-associated increase in HPC spine density was unchanged by Blebb treatment. However, METH-associated memory was disrupted when animals were tested a second time (24 hours) after intra-HPC infusion, suggesting an effect on reconsolidation. Indeed,

Blebb disrupted METH-associated memory when delivered into the HPC immediately after a brief memory recall session (5 mins), but not a full test session (15 mins), suggesting a brief time window for disruption of reconsolidation-based actin dynamics. Taken together these results build upon our previous work on nonmuscle myosin II, shedding light on a second mechanism by which Blebb functions to disrupt drug-associated memory. This suggests that, if combined with retrieval, nonmuscle myosin II inhibition may prove to be a powerful therapeutic approach by disrupting storage of the portion of METH-associated memory trace stored in the AMY (no retrieval required) and reconsolidation of the portion of the memory trace stored in the HPC.

Disclosures: S.B. Briggs: None. **E.J. Young:** None. **A.M. Blouin:** None. **G. Rumbaugh:** None. **C.A. Miller:** None.

Poster

541. Drug Addiction: Learning and Memory

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Topic: G.08. Drugs of Abuse and Addiction

Title: Delayed protein expression in the mPFC in a critical time window is necessary for late consolidation of cocaine incubation

Authors: *C. CHEN;
The Sixth Hosp. of Peking Univ., Beijing City, China

Abstract: Introduction: One of the challenges of cocaine addiction is the incubation in which relapse to drug use can occur after prolonged withdrawal. Although several brain regions and molecular mechanisms were involved in this process, the underlying mnemonic mechanisms are still unknown. Long-term memory persistence requires a late protein synthesis-dependent phase, even many hours after memory acquisition. BDNF is a crucial protein synthesis product that has emerged as a potent molecular mediator for synaptic plasticity. Here we explored whether delayed BDNF expression during the time window of late consolidation in mPFC regulate cocaine incubation **Methods:** Rats tested the double-wave of protein synthesis expression after 10 d self-administration (SA) training. Rats were harvested brain at 7 different time points after last training for expression analysis including 0.5 h, 1 h, 9 h, 12 h, 18 h, 24 h and 36 h. We detected brain activity after SA training by analyzing c-fos expression in several brain regions, including prelimbic cortex (PrL), infralimbic cortex (IL), basolateral amygdale (BLA) and central amygdala (CeA). Then, we explored the effects of microinjection of the protein synthesis inhibitor anisomycin in target brain region on drug craving after 30 d withdrawal. Next, we will regulate BDNF-TrkB-ERK signaling pathway activity to further confirm whether this pathway

plays a crucial role in the late consolidation of cocaine craving. **Results:** Our preliminary experiment showed the early and late protein expression waves in PrL and IL after 10 d SA training. In the PrL, the peak of c-fos expression presented on 0.5 h, 1 h and 18 h after last SA training and only 0.5 h and 24 h were found in IL. In the BLA, only the early wave of protein expression was existed, and no significant difference was found in CeA. Cocaine incubation was sensitive to protein synthesis inhibition at 18 h after the last SA training in the PrL, and the active nosepokes were decreased in 30 d withdrawal test but not 2 d withdrawal test. **Conclusion:** These results indicate that delayed protein expression in a critical time window is involved in late consolidation of cocaine incubation.

Disclosures: C. Chen: None.

Poster

541. Drug Addiction: Learning and Memory

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Topic: G.08. Drugs of Abuse and Addiction

Support: Whitehall Foundation Research Grant award (APP131146, AJR), NIEHS

Title: Epigenetic regulation of the FosB gene in hippocampal-dependent behaviors

Authors: *P. A. GAJEWSKI[1], A. L. EAGLE[2], E. A. HELLER[3], I. MAZE[4], A. J. ROBISON[2]; [1]Genet., [2]Physiol., Michigan State Univ., East Lansing, MI; [3]Pharmacol., Univ. of Pennsylvania, Pennsylvania, PA; [4]Neurosci., Mount Sinai Sch. of Med., New York, NY

Abstract: Drug addiction results in part from maladaptive learning, including the formation of strong associations between the drug and the environment and circumstances of its use. However, the patterns of gene regulation critical for this learning remain unknown. Consolidation of explicit memories occurs through synaptic plasticity in the hippocampus, and some of the molecular mechanisms of this process are well characterized, but epigenetic regulation underlying changes in hippocampal gene expression and the distribution of gene expression through the hippocampus is poorly understood. The transcription factor ΔFosB is an important arbitrator of activity-dependent gene expression, and our group has recently demonstrated that its expression in hippocampus is critical for learning. Previous studies demonstrate that drugs of abuse also upregulate ΔFosB in hippocampus, but the mechanism of its induction by cocaine in hippocampus and its role in hippocampus-dependent cocaine responses is unknown. We are currently determining whether the expression pattern of ΔFosB differs throughout hippocampal subregions in response to cocaine-related learning behaviors, as it

appears to be uniquely induced in the CA1 subregions in response to spatial learning. Furthermore, we use chromatin immunoprecipitation to demonstrate that dimethylation of lysine 9 at histone H3, a repressive histone modification, is decreased at the *FosB* gene promoter in hippocampus after chronic exposure to cocaine and general learning. We show that locus specific modification of this histone mark in dorsal hippocampus is sufficient to impair learning and memory. Experiments are ongoing to determine whether these locus-specific histone modifications impact drug-related behaviors, as well. These findings collectively suggest that specific salient stimuli, such as formation of drug-environment associations, induce epigenetic changes in the hippocampal *FosB* gene promoter that regulate ΔFosB induction, which in turn may control the transcription of genes that underlie hippocampal cell function, plasticity, and cocaine-related learning.

Disclosures: P.A. Gajewski: None. **A.L. Eagle:** None. **E.A. Heller:** None. **I. Maze:** None. **A.J. Robison:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: This research was supported financially by the Psychology Department at Dickinson College.

Title: Increasing CS duration facilitates extinction and blunts context-specific sensitization in mice

Authors: *A. S. RAUHUT, J. BRITTON, A. YOUNG;
Psychology, Dickinson Col., Carlisle, PA

Abstract: Associative learning processes have been proposed to contribute to behavioral sensitization; however, previous research has shown that extinction does not alter expression of behavioral sensitization in rodents. The duration of conditioned stimulus (CS) exposure has been shown to be an important variable in determining the rate and robustness of extinction in the Pavlovian conditioning literature. Thus, the present experiment examined the effect of varying the CS duration on the rate of extinction of conditioned hyperactivity and the subsequent expression of behavioral sensitization in mice. The experiment consisted on 3 phases: acquisition, extinction and test for behavioral sensitization. During acquisition, male, Swiss Webster mice (n = 6/group) received an injection (subcutaneous, s.c.) of methamphetamine (1.0

mg/kg; paired) or vehicle (saline; unpaired) on 4 alternating, 30-minute locomotor activity sessions (Chamber Days). On the 4 intervening sessions (Home Cage Days), paired and unpaired mice received vehicle or methamphetamine, respectively, in their home cages. Following the acquisition phase, the extinction phase began and lasted 8 sessions. At this time, paired and unpaired mice received an injection of vehicle and placed in the locomotor activity chambers for either 30 or 120 minutes. Two control groups (Paired-Rest and Unpaired-Rest) were included that did not undergo extinction and remained in their home cages for the entirety of this phase. The test for behavioral sensitization occurred 24 hours after the last extinction session. All mice received an injection of a challenge methamphetamine dose (0.5 mg/kg) and tested for a 120-minute period in the locomotor activity chambers. It was found that paired mice that received 120-minute extinction sessions extinguished faster and showed a less robust sensitization response to methamphetamine compared to paired mice that underwent only 30-minute extinction sessions. These results add further support for the role of associative learning processes in behavioral sensitization and suggest that factors (e.g., CS duration) that promote extinction may alter the expression of behavioral sensitization.

Disclosures: **A.S. Rauhut:** None. **J. Britton:** None. **A. Young:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Title: *In vivo* calcium imaging to assess the role of prelimbic cortex neuronal ensembles in encoding reinstatement of palatable food seeking in rats.

Authors: *R. MADANGOPAL, D. CAPRIOLI, B. LIANG, G. BARBERA, B. T. HOPE, Y. SHAHAM, D.-T. LIN;
Natl. Inst. On Drug Abuse IRP, Baltimore, MD

Abstract: Background & rationale: *In vivo* imaging in awake behaving rats provides a dynamic, spatio-temporal view of task-related ensemble activity during the course of learning. Previous studies using the rat reinstatement model have implicated the prelimbic cortex in relapse to palatable food-seeking induced by non-contingent pellet priming. However, the underlying neuronal ensemble activity in the prelimbic cortex that encodes pellet priming-induced reinstatement is unknown.
Technique: We developed a miniature epifluorescent microscope for imaging calcium dynamics in awake behaving rats. We also optimized protocols for implantation and coupling with gradient

index (GRIN) lenses for long-term imaging in prelimbic cortex of awake behaving rats.
Behavioral procedure: We trained rats that were food restricted to 75% *ad lib* feeding level to lever press for a palatable food pellet reward (fixed ratio 1 schedule; 1 pellet reward delivered after presentation of a 10s discrete cue) in a modified trial-based design (60 trials/session; 60-s lever availability/trial; variable inter-trial interval). Next, we extinguished the rats' lever-pressing in the presence of the discrete cue. We then tested the rats under extinction conditions for pellet priming-induced reinstatement of palatable food seeking.
Results: We observed reliable food self-administration and extinction of food-reinforced responding in the trial-based procedure. We also observed robust pellet-primed reinstatement under extinction conditions.
Ongoing studies: We are currently recording task-specific *in vivo* neuronal activity in prelimbic cortex during our trial-based task to identify and monitor neuronal ensembles that encode food-seeking behavior.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH grant R21-DA038377

NIH grant R01-DA031852

Title: Genetic depletion of D2 receptors in dopamine neurons increases locomotor sensitivity to cocaine and impairs reversal learning in mice

Authors: J. LINDEN[1], A. S. JAMES[1], C. MCDANIEL[2], C. GOODMAN[2], *J. D. JENTSCH[1]; [1]Binghamton Univ., Binghamton, NY; [2]Univ. of California at Los Angeles, Los Angeles, CA

Abstract: Neuroimaging studies in rodents and non-human and human primates have all revealed that relatively low striatal dopamine D2-like receptor binding potential is associated with poor impulse control and with vulnerability for addiction-related behaviors. These studies cannot, however, disambiguate the roles for various pools of D2 receptors found in the striatum (e.g., those expressed on medium spiny striatopallidal neurons vs. on dopamine-releasing nerve terminals) in these behavioral outcomes. To clarify the role of the latter pool - namely, D2

autoreceptors - we studied mice carrying a conditional DRD2 gene, with or without Cre-recombinase expressed under the transcriptional control of the dopamine transporter gene locus (DAT-DRD2flox, N=19 and WT-DRD2flox, N=21). These mice were tested for response to cocaine in locomotor activity chambers, and spatial reversal learning was assessed in operant boxes. As predicted, compared to WT-DRD2flox mice, DAT-DRD2flox animals demonstrated heightened sensitivity to the locomotor stimulating effect of cocaine (10 mg/kg, i.p.), confirming previous research using a similar genetic model. In the spatial reversal learning task, DAT-DRD2flox mice were slower to reach a learning criterion, committed higher numbers of anticipatory responses and had difficulty sustaining a prolonged nose poke response, all measurements related to defective response inhibition. Rate of learning of the initial discrimination and latencies to collect rewards, to initiate trials and to produce a response were unaffected by genetic deletion of D2 autoreceptors, discarding possible motor and motivational factors. Together, these findings confirm the role of D2 autoreceptors in reversal learning and suggest a broader involvement in behavioral inhibition mechanisms.

Disclosures: J. Linden: None. **A.S. James:** None. **C. McDaniel:** None. **C. Goodman:** None. **J.D. Jentsch:** None.

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Program#/Poster#: 541.17/HHH19

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH DA033404

Washington State University Alcohol and Drug Abuse Research Program

Title: Suppression of 50kHz ultrasonic vocalizations in rats as a sensitive measure of memory in cocaine-induced conditioned place preference

Authors: *P. S. KUDVA, K.-A. REYES, B. SORG;
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Abstract: Reconsolidation of a memory is the process of reactivating a memory and then re-stabilizing it, thereby strengthening it. When a memory is reactivated, it can be disrupted using specific pharmacological agents that weaken it. Rats emit ultrasonic vocalizations (USVs) in response to various stimuli, and the emission of frequency-modulated 50kHz USVs (FM50 USVs) is believed to be associated with positive affective states. Here we examined the impact

of the alpha-1 adrenergic antagonist, prazosin, on the ability to disrupt the reconsolidation of memories associated with cocaine in a cocaine-induced conditioned place preference (CPP) task. We tested the hypothesis that USVs would be suppressed after disruption of reconsolidation by prazosin, and that this affective measure would be a more sensitive indicator of memory than traditional CPP (motor) behavior. During CPP training, rats were confined to one side of the CPP box and received an IP injection of either cocaine or saline on alternating days for eight days. On the CPP Test day, rats were allowed open access to both sides of the box without any injection. Rats demonstrated increased FM50 USVs on the cocaine- vs. saline-paired side. Their memory was then reactivated the next day by confinement in the cocaine-paired chamber for 15 min followed an IP injection of prazosin or vehicle. The following day, rats were allowed open access to both sides of the box. While CPP behavior was reduced in prazosin-treated rats, FM 50 USVs USVs were still elevated on the cocaine-paired side equal to that of vehicle controls. In addition, a separate cohort of rats that did not demonstrate significant CPP behavior on the CPP Test day still demonstrated higher FM50 USVs on the cocaine-paired side. These results indicate that FM50 USVs may be a more sensitive indicator of memory than CPP behavior.

Disclosures: **P.S. Kudva:** None. **K. Reyes:** None. **B. Sorg:** None.

Poster

541. Drug Addiction: Learning and Memory

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PSI 2015-68600-P

Predoc/2014/11

Title: Cerebellar and medial prefrontal deactivations effects on the acquisition of cocaine-induced preference conditioning

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Abstract: The cerebellum has traditionally been overlooked in addiction research, despite growing evidences that suggest a role for it in many of the brain functions affected in addicts. Recent studies have demonstrated that the cerebellum mediates the consolidation of emotional

memories; the development of reward-induced learning; and the persistence of procedural memory. Moreover, clinical data suggest that lesions and pathological conditions might reorganize functions of the prefrontal-cerebellar circuitry. Recently, we have found two cerebellar hallmark signatures of conditioned preference for cocaine: an increase in cFOS expression in cells at the apex of the granular layer and stronger expression of the perineuronal nets (PNNs) surrounding Golgi interneurons in the same region. In the present study, we analysed the effect produced by the impairment of these prefronto-cerebellar networks in the acquisition of cocaine-induced preference conditioning. We evaluated the effects of different mPFC and cerebellar deactivations in rats before starting the conditioning training to acquiring preference towards an olfactory stimulus paired with cocaine. Two groups of rats were subjected before training to a temporary prelimbic or infralimbic inactivation by lidocaine. Other two groups were treated with quinolinic acid for a permanent lesion in two different regions of the posterior cerebellar vermis. In this work, we report that either the inactivation of infralimbic cortex or the lesion of the cerebellar apex of the vermis significantly increased the percentage of animals acquiring conditioned preference for cocaine. Therefore, our findings showed that the development of cocaine-induced conditioned preference is promoted when activity in either the infralimbic cortex or the apex of the posterior vermis decreases. A simultaneous inactivation of both sites prevented this effect. Opposite results were found after either prelimbic impairment or ventral cerebellar lesion. The results suggest that the mPFC cortex and cerebellum work together on the acquisition of drug-related emotional memories. To study the anatomical network behind this process retrograde and anterograde tracers were injected in the same site of cerebellar cortex and mPrf cortex where lesions were placed.

Disclosures: **I. Gil Miravet:** None. **M. Carbo Gas:** None. **J. Moreno Rius:** None. **F.E. Olucha Bordonau:** None. **M. Miquel:** None.

Poster

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GAUK748216

Title: The role of ghrelin in morphine and methamphetamine induced conditioned place preference in rats

Authors: *M. SUSTKOVA[1], T. HAVLICKOVA[1], N. PUSHKINA[2], C. CHARALAMBOUS[2], M. LAPKA[1];
[1]Dep Pharmacol. Third Fac. of Med. Charles Univ. in Prague, Charles Univ. In Prague, Praha 10, Czech Republic; [2]Dep Addictology, First Fac. Med. Charles Univ., Prague 2, Czech Republic

Abstract: Aims: An increasing number of studies over the past few years have demonstrated ghrelin´s participation in alcohol, cocaine and nicotine abuse. However, the role of ghrelin in opioid and methamphetamine effects has rarely been examined. The aim of the present study in rats was to ascertain whether a ghrelin antagonist (JMV2959) was able to inhibit morphine induced and methamphetamine induced conditioned place preference (CPP) and also to prevent the methamphetamine-induced place conditioning process. Methods: In the biased CPP model, rats were conditioned for 8 days with morphine (10 mg/kg s.c.) or methamphetamine (5 mg/kg i.p.). On the experimental day, JMV2959 (3 and 6 mg/kg i.p.) or saline were administered 20 min before testing. In another experiment JMV2959 (3 and 6 mg/kg i.p.) was administered always together with methamphetamine during the conditioning process. There were 14 rats in each group of morphine and 8 rats in groups of methamphetamine experiments. Results: One way ANOVA followed by Holm Shidak test revealed significant and dose dependent reduction of morphine-induced as well as methamphetamine induced CPP after acute JMV2959 pretreatment (3 and 6 mg/kg). Pretreatment with JMV2959 (3 and 6 mg/kg) during the conditioning process significantly and dose-dependently reduced the methamphetamine induced CPP. Conclusion: Ghrelin secretagogue receptors (GHS R1A) appear to be significantly involved in the morphine induced and methamphetamine induced conditioning associated with the reward processing.

Disclosures: M. Sustkova: None. **T. Havlickova:** None. **N. Pushkina:** None. **C. Charalambous:** None. **M. Lapka:** None.

Poster

541. Drug Addiction: Learning and Memory

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Topic: G.08. Drugs of Abuse and Addiction

Support: Whitehall Foundation Research Grant (APP131146)

NIEHS Training Grant (T32-ES725525)

2014 NARSAD Young Investigator Award

Title: Activity-dependent transcriptional regulation of hippocampal projections in fear/anxiety and cocaine reward

Authors: *A. L. EAGLE[1], C. E. MANNING[1], P. A. GAJEWSKI[1], R. ACHARGUI[1], L. A. GRON[1], F. M. BOYCE[2], R. L. NEVE[3], A. J. ROBISON[1];
[1]Dept. of Physiol., Michigan State Univ., East Lansing, MI; [2]Dept. of Neurol., Massachusetts Gen. Hosp., Cambridge, MA; [3]Viral Gene Transfer Core, MIT, Cambridge, MA

Abstract: Drug seeking, a hallmark of addiction, is mediated by neurobiological adaptations in the reward circuitry of the brain. In particular dysfunctional regulation of reward regions, like nucleus accumbens (NAc), promotes the development of drug addiction, and evidence suggests this is mediated by direct glutamatergic projections from ventral hippocampus (vHPC) to NAc. In addition, the vHPC is important for regulating fear and anxiety behavior via direct projections to the amygdala (Amy). These projection neurons may also undergo significant functional adaptations after learning, stress, or exposure to drugs of abuse, such as cocaine, that may depend, in part, from activity-dependent alterations in gene expression. However, the mechanisms underlying vHPC projection-specific changes in gene expression are not understood. ΔFosB, a truncated splice product of the *FOSB* gene, is a *chronic* activity-dependent transcription factor that we have recently demonstrated is critical for hippocampus-dependent learning. We therefore sought to determine the role of ΔFosB in vHPC projections to NAc and Amy and subsequent drug seeking (e.g. drug reward) and fear/anxiety behaviors. To this end, we developed a novel dual-viral CRISPR-Cas9 technique to selectively silence the *FOSB* gene in vHPC projections to NAc or Amy in adult mice. We first demonstrate here that non-specific ΔFosB inhibition in the ventral HPC (vHPC) impairs cocaine conditioned place preference (CPP), which measures the expression of drug reward, and avoidant learning, both of which are known to be mediated by the function of NAc and Amy, respectively. Additionally these same behaviors induce ΔFosB in vHPC neurons in a projection-specific manner. We then examined the effects of selective silencing of *FOSB* in specific vHPC projections on these same behaviors. We found that *FOSB* in vHPC-NAc projections is critical for the expression of cocaine CPP, but not avoidant learning. Conversely, *FOSB* KO in vHPC-Amy projections impaired avoidant learning and reduced anxiety-like behavior, but did not affect expression of cocaine CPP. These findings suggest that ΔFosB regulates projection-specific vHPC neuron functional adaptations via changes in gene expression, thereby driving cocaine-dependent behaviors and stress-evoked behaviors, such as fear & anxiety. Furthermore, they suggest that ΔFosB in vHPC projections may be important in drug addiction and stress-related psychiatric disorders, such as posttraumatic stress disorder and depression, serving as a viable target for drug development for these disorders.

Disclosures: **A.L. Eagle:** None. **C.E. Manning:** None. **P.A. Gajewski:** None. **R. Achargui:** None. **L.A. Gron:** None. **F.M. Boyce:** None. **R.L. Neve:** None. **A.J. Robison:** None.

Poster

541. Drug Addiction: Learning and Memory

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Program#/Poster#: 541.21/HHH23

Topic: G.08. Drugs of Abuse and Addiction

Title: Identifying functional alterations in neuronal ensembles of medial prefrontal cortex activated during food self-administration in rats

Authors: *L. R. WHITAKER, B. L. WARREN, T. C. HARTE, J. M. BOSSERT, K. B. MCPHERSON, J. BEIDEL, Y. SHAHAM, A. BONCI, B. T. HOPE;
Natl. Inst. on Drug Abuse Intramural Res. Program, Baltimore, MD

Abstract: Learned associations between environmental stimuli and rewards drive goal-directed learning and motivated behavior. These associations are thought to be encoded by specific patterns of sparsely distributed neurons called neuronal ensembles that are determined by selective activation of reward-predictive stimuli. The question remains as to how neurons in these ensembles are functionally altered during learning, and which of these changes encode learned associations. The objectives of our study were to identify ensembles of neurons strongly activated during food self-administration, and then to determine functional alterations specific to these neuronal ensembles. Training took place over a period of 10 days. During each training session, rats were allowed to lever press for food pellets in the self-administration chambers for one hour. Rats formed an association between lever pressing and food reward over the course of ten days of training. Immunohistochemical analysis was performed on days 1, 3 and 10 of training. Preliminary data show induction of Fos expression on training day 1 that diminishes by day 10. Additionally, whole cell recordings were performed in *Fos-GFP* transgenic rats on days 1 and 10 of training to identify functional alterations that contribute to operant learning. In these rats, strong neuronal activation activates the *Fos* promoter and drives expression of GFP. No changes in intrinsic excitability were observed in FosGFP+ neurons over the course of training. However, there was a decrease in excitability in the FosGFP- neurons from day 1 to day 10 of training. Future studies will identify the mechanism underlying this alteration in membrane excitability following operant learning. This work was supported by NIDA Intramural Research Program.

Disclosures: L.R. Whitaker: None. **B.L. Warren:** None. **T.C. Harte:** None. **J.M. Bossert:** None. **K.B. McPherson:** None. **J. Beidel:** None. **Y. Shaham:** None. **A. Bonci:** None. **B.T. Hope:** None.

Poster

541. Drug Addiction: Learning and Memory

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Topic: G.08. Drugs of Abuse and Addiction

Support: This research was supported by the Intramural Research Program of the NIH, NIDA

Title: Fos expression in the infralimbic cortex following extinction of cocaine seeking.

Authors: *B. L. WARREN, M. P. MENDOZA, F. SOTO, D. CAPRIOLI, M. VENNIRO, L. R. WHITAKER, R. MADANGOPAL, Y. SHAHAM, B. T. HOPE;
Natl. Inst. on Drug Abuse Intramural Res. Program, Baltimore, MD

Abstract: Background: We recently found that Fos-expressing neuronal ensembles in the infralimbic cortex encode operant extinction of food seeking. Here we tested whether this finding generalize to extinction of operant cocaine seeking.
Methods: We trained Long-Evans rats to lever press for infusions of cocaine for 14 days days (3 h/day; 1.0 mg/kg/infusion on days 1-7 and 0.5 mg/kg/infusion on days 8-14). We subsequently exposed rats to 0, 2, or 7 daily extinction sessions (3 h/day) to assess Fos expression at three different time points following extinction training. The next day, we exposed all rats to a short 15 min test under non-reinforced conditions to induce Fos. We then assessed Fos immunoreactivity in the infralimbic cortex.
Results: Rats increased their lever pressing for cocaine infusions over the course of self-administration training. Furthermore, rats doubled their lever pressing when the dose of cocaine per infusion was cut in half. On test day, rats decreased their lever pressing following both 2 and 7 prior extinction sessions. We found maximal Fos expression in the infralimbic cortex of rats exposed to 2 prior extinction sessions.
Conclusions: Increased Fos expression in the infralimbic cortex suggests that neuronal ensemble activity in this area encode extinction of cocaine seeking. We currently test this hypothesis using the Daun02 inactivation procedure.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: Department of Psychology, University of Evansville

Title: The addictive potential of artificial sweeteners

Authors: *L. A. BECKER, W. MILLER, V. DEERE, N. BEDI, K. MAYES, K. SHEETS; Psychology, Univ. of Evansville, Evansville, IN

Abstract: This study explored the potential for artificial sweetener addiction in comparison to the potential for sugar addiction, using rats as an animal model. Research focused on adolescent rats, such that artificial sweetener addiction might be correlated with the rise in obesity among teenagers (Fowler et al., 2008; Ogden et al., 2012). Rats were exposed to one of six conditions, with four groups having a sweetener added to their water supply that varied in type and concentration: 10% sucrose, 30% sucrose, 0.4% sucralose, and 0.8% saccharin. An additional group served as a negative control (water) and a final group acted as a positive control (water), given daily intraperitoneal injections of 1.6 mg/kg nicotine, allowing for the contrasting of sweetener addiction to nicotine addiction. Addiction was achieved via food and water deprivation 12 hours daily for a period of eight weeks, thereby inducing bingeing and withdrawal behaviors. This addiction was then quantified by measuring withdrawal symptoms brought on by the injection of naloxone, an opioid antagonist, prior to a series of three tests: open field, elevated-plus maze, and Porsalt forced swim. Rats in the artificial sweetener conditions spent less time in the center of the open field than rats in the sugar condition and rats in either condition swam a lesser distance than negative control rats in the Porsalt forced swim test. These data support that artificial sweeteners are at least equally as addictive as sugar, but that neither sweeteners are as addictive as nicotine. Thus, it is hypothesized that increased consumption of artificial sweeteners is correlated with the rise in teenage obesity.

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Poster

541. Drug Addiction: Learning and Memory

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Topic: G.08. Drugs of Abuse and Addiction

Title: Oral self-administration of caffeine in rats.

Authors: *C. A. BRADLEY, E. A. WILLIAMS, M. I. PALMATIER;
East Tennessee State Univ., Johnson City, TN

Abstract: Caffeine is the most consumed psychoactive drug in the world and functions as a reinforcer in humans, but no previously published study has established reliable caffeine self-administration in non-humans. However, our laboratory has found that caffeine is a robust 'reinforcement enhancer' - it increases responding for non-drug reinforcers such as sucrose and saccharin. We have recently leveraged this effect to establish intravenous self-administration caffeine. When rats earned caffeine infusions and a reinforcing taste stimulus (0.2% saccharin) for making an operant response, they increased their responding relative to a group receiving the taste stimulus alone. The objective of the present studies was to establish a more veridical model in which oral caffeine is consumed in a vehicle that also contained the non-drug reinforcer. Because of the potent bitter taste of caffeine, we predicted that a masking stimulus (decaffeinated coffee, 0.5% w/v) would enhance oral caffeine intake at higher concentrations. Forty rats were shaped to lever press for 0.1 ml presentations of saccharin (0.2% w/v) in a liquid dipper under a progressive ratio (PR) schedule of reinforcement. Rats were subsequently assigned to one of two drug conditions: vehicle alone (-) or caffeine included in the vehicle (+), and one of three vehicle conditions: saccharin alone (S+ or S-), saccharin with decaffeinated coffee (SD+ or SD-), or water (W+ or W-). After initial acquisition with a moderate concentration of caffeine (2.5 mg/ml) additional concentrations were tested (3.5, 5.0, and 7.0 mg/ml) to describe the relationship between caffeine dose and operant behavior. All sessions ended after rats reached a 20 minute breaking point (20 min with no further reinforcement earned). The S- and DS- groups did not differ in their motivation to obtain the vehicle solutions, indicating no difference in reinforcing effects of the vehicles. However, caffeine robustly increased responding in the S+ and DS+ groups, confirming that oral caffeine is self-administered by rodents. Decaffeinated coffee did not enhance responding at any concentration, suggesting that a masking flavor is not needed to establish or promote oral caffeine intake. The effects of caffeine were also dose-dependent, with peak concentrations between 2.5 and 3.5 mg/ml. Oral caffeine in water (W+) reduced intake relative to the vehicle control (W-).These findings are the first to clearly establish that caffeine is self-administered orally in concentrations that do not have primary reinforcing effects in rodents.

Disclosures: **C.A. Bradley:** None. **E.A. Williams:** None. **M.I. Palmatier:** None.

Poster

541. Drug Addiction: Learning and Memory

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Topic: G.08. Drugs of Abuse and Addiction

Support: Whitehall Foundation Research Grant

NARSAD Young Investigator Award (22774)

NIH Grant 5T32NS044928-12

Title: DeltaFosB expression in the hippocampus regulates behavior in the social defeat model of depression

Authors: ***C. MANNING**, A. L. EAGLE, P. A. GAJEWSKI, M. MAZEI-ROBISON, A. ROBISON;
Michigan State Univ., East Lansing, MI

Abstract: Stressful and traumatic experiences can contribute to mood disorders in some individuals while others are resilient. Though we know that the hippocampus plays a crucial role in stress responses, it is unknown how changes in hippocampal function, particularly at the level of gene expression, may drive resilience to stress. We have recently described a critical role for hippocampal expression of the transcription factor ΔFosB, encoded by the *FosB* gene, in general learning, but its role in hippocampal control of stress responses is unknown. The mouse chronic social defeat stress (CSDS) model of depression has both pharmacological and face validity, and has the added advantage of generating both susceptible and resilient animals, and we have therefore used this model to investigate the role of hippocampal ΔFosB in stress resilience. Here we show that animals with *FosB* developmental knockout (KO) restricted to the dentate gyrus and CA3 subregions of the hippocampus show both increased susceptibility to stress and reduced rates of antidepressant-induced neurogenesis. Moreover, using a novel dual-virus CRISPR system, we show that silencing of the *FosB* gene in adulthood in specific hippocampal cells projecting to nucleus accumbens increases susceptibility to CSDS. This result provides a molecular mechanism for recent reports that glutamatergic hippocampal afferents to nucleus accumbens regulate susceptibility to CSDS, and acute stimulation in these projections result in a susceptible phenotype (Bagot 2015). Our data also implicate ΔFosB in regulating cellular excitability, as overexpression of ΔFosB in these CA1 projection neurons results in reduced

excitability and irregular action potentials. Taken together, these data suggest that ΔFosB expression in response to stress regulates hippocampal cells projecting to NAc to promote resilience. We are currently investigating potential downstream targets of ΔFosB in the hippocampus and ΔFosB's role in hippocampal neurogenesis.

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Poster

541. Drug Addiction: Learning and Memory

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Program#/Poster#: 541.26/HHH28

Topic: G.08. Drugs of Abuse and Addiction

Support: R01 DA037216-02

IBX000741B, VA

Title: Assessing acid-sensing ion channels in instrumental learning behavior

Authors: *S. M. ALAM[1], A. GHOBBEH[1], R. J. TAUGHER[1], R. FAN[1], R. T. LALUMIERE[2], J. A. WEMMIE[1];
[1]Psychiatry, Univ. of Iowa Dept. of Psychiatry, Iowa City, IA; [2]Psychology, Univ. of Iowa Dept. of Psychology, Iowa City, IA

Abstract: Acid-sensing ion channel-1A (ASIC1A) is a proton-gated cation channel activated by extracellular acidosis. ASIC1A is expressed throughout the brain including the nucleus accumbens and amygdala, is localized to dendritic spines, and has been implicated in learning, memory, and synaptic plasticity. Recent data suggest roles for ASIC1A in cocaine- and morphine-seeking behaviors including operant drug self-administration. These observations motivated us to test whether ASIC1A is involved in instrumental learning to non-drug rewards. We trained *Asic1a*$^{+/+}$ and *Asic1a*$^{-/-}$ mice to press a lever for various rewards in an operant behavioral task. We then assessed motivation using fixed-ratio 1 and progressive ratio reinforcement paradigms. In both paradigms, *Asic1a*$^{-/-}$ mice performed normally, suggesting normal task acquisition and motivation. We next removed the reward to test extinction learning. Both *Asic1a*$^{+/+}$ and *Asic1a*$^{-/-}$ mice extinguished normally. These data suggest that ASIC1A disruption does not cause deficits in instrumental reward learning. Furthermore, the previously identified role of ASIC1A in reward behavior may be specific to drugs of abuse.

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Poster

541. Drug Addiction: Learning and Memory

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA020098

NIH Grant P60 AA06420

Title: Withdrawal from alcohol induces transient microstructure changes in grey and white matter structures in the rodent prefrontal cortex

Authors: *S. S. SOMKUWAR[1,2], E. VILLALPANDO[3], M. SCADENG[3], M. FANNON[2], C. MANDYAM, 92129[2];
[1]San Diego, CA; [2]Veterans Affairs San Diego Hlth. Syst., San Diego, CA; [3]Univ. of San Diego, San Diego, CA

Abstract: White matter abnormalities are observed in heavy consumers of alcohol. Recent morpho-anatomical studies also reveal decreases in grey matter volume in the frontal lobe of individuals addicted to alcohol. Diffusion tensor imaging (DTI) is a magnetic resonance imaging technique that reveals valuable information regarding microstructural architecture of the brain, as such, decreased fractional anisotropy (FA) is associated with demyelination or dysmyelination. The current study investigated temporal changes in DTI measures during protracted abstinence from alcohol in a rat model of alcohol addiction. Adult male rats were subject to seven weeks of chronic intermittent ethanol exposure (CIE) paradigm where rats breathed air containing alcohol vapor for 14 hours/day that produced blood alcohol levels of 150-250 mg/dl. DTI was conducted 24 hours, 7 days, 21 days or 42 days after cessation of the seven-week CIE procedure in isoflurane anesthetized CIE rats, and in age-matched alcohol-naïve controls (n=4/group). FA and mean diffusivity (MD) were measured in specific regions of interest (ROIs) in the prefrontal cortex, specifically, in infralimbic, prelimbic, anterior cingulate and motor cortices and in the corpus callosum. FA and MD values, expressed as % of control, at various time-points after cessation of CIE were compared in the individual ROIs using separate one-way ANOVAs. In the corpus callosum, prelimbic and infralimbic cortices, FA was decreased at 21 days compared to 24 hours post CIE, and was increased at 42 days compared to 21 days; no other differences were revealed between the various time-points. In the anterior cingulate and motor cortices, FA was

decreased at 7 days and 21 days compared to 24 hours and was increased at 42 days compared to 7 days post CIE. In corpus callosum as well as in anterior cingulate, prelimbic and infralimbic cortices, MD was decreased at 21 days compared to 24 hours post CIE, no other differences were revealed in MD in these regions. In contrast, MD was decreased at 7 days, 21 days as well as 42 days post CIE compared to 24 hour time-point in the motor cortex. Thus, evidence of transient demyelination was observed in both the grey and white matter regions of the prefrontal cortex of rats withdrawn from CIE. Brain region differences were noted in the persistence of these changes, such that more permanent myelin loss was revealed in the motor cortex compared to the other regions. Taken together, the results in the rat model are consistent with observations from human imaging studies, thereby providing validation for a translationally important model to study causal link between excessive alcohol intake and prefrontal myelin neuropathology.

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Poster

542. Motivation and Economic Processes

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Program#/Poster#: 542.01/HHH30

Topic: G.02. Motivation

Support: NIH R01(DA038106)

Title: Distinct neural processes for appetitive and informational rewards

Authors: *E. S. BROMBERG-MARTIN[1,2], J. MEREL[1,2], T. C. BLANCHARD[3,4], B. Y. HAYDEN[4];
[1]Columbia Univ., New York, NY; [2]Kavli Inst. for Brain Sci., New York, NY; [3]Harvard Univ., Cambridge, MA; [4]Univ. of Rochester, Rochester, NY

Abstract: When we are uncertain about future rewards, we often have a strong desire to seek out informative sensory cues that will tell us what our future holds. This information seeking behavior is common in our everyday lives, but is not accounted for by current theories of reinforcement learning and decision making. In fact, we often seek information even when it has no 'objective' value in terms of gathering rewards, as if the information has an intrinsic, 'subjective' value of its own. Here we test two major proposals that have been put forward to explain information seeking. According to the two-process theory, the brain has two distinct motivational processes: one for valuing primary rewards (such as food and water) and one for valuing information about those rewards. According to the single-process theory, however, both

behaviors stem from a single process tied to primary rewards; information seeking is explained as a mere side-effect of suboptimal, nonlinear distortions in learning of cue values. We present neural and behavioral evidence for the two-process theory. We gave monkeys a choice between gambles that differed in two dimensions: the amount of water reward at stake, and the chance to see informative cues that indicated the gamble's outcome several seconds in advance of reward delivery. Behaviorally, we show that monkeys assign high value to information, paying more than 20% of their water reward in exchange for informative cues. We show using theory and model-fitting that their behavior is better explained by two-process than one-process models. Neurally, we recorded from cells in the orbitofrontal cortex (OFC) which have been hypothesized to represent the subjective value of choice alternatives. Indeed, OFC neurons signaled the water reward and cue informativeness of each gamble. However, strikingly, OFC neurons signaled water and informativeness in uncorrelated manners, resembling the distinct processes in two-process models. Thus, our data suggest that information-seeking is not merely a side-effect of seeking primary rewards; the brain appears to value information in its own right.

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Poster

542. Motivation and Economic Processes

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Program#/Poster#: 542.02/HHH31

Topic: G.02. Motivation

Support: Alfred P. Sloan Foundation

R00 MH099093

Title: Seeking information in a social reward donation task

Authors: *J. A. JOINER[1], S. W. C. CHANG[2];
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Abstract: Both primary reinforcers, such as food, and more abstract reinforcers, such as information, strongly influence our behaviors. Like humans, non-human primates find information about upcoming rewards to be reinforcing regardless of whether or not that information influences their tangible reward outcomes (Bromberg-Martin & Hikosaka, 2011). However, whether or not monkeys find reward information about others similarly reinforcing has yet to be explored. While we know that rhesus macaques are curious about the value of own

rewards, we do not know if they are also curious about the reward values of others and if their information seeking behavior captures the underlying preferences they have about the rewards at stake during social interactions.
To explore social curiosity and how it is related to social reward sharing preferences, we utilize a modified version of the juice donation game in which an actor monkey chooses to allocate juice to himself, another monkey, both himself and the other monkey, or no one at all. Critically, after choosing any of these options, but prior to the reward delivery, the monkeys were presented with an opportunity to reveal the magnitude of the upcoming reward in each of the different reward scenarios at a specified cost. They were able to either opt in or opt out of seeking the reward value information by fixating on an occluder for different lengths of time in order to uncover a reward magnitude cue indicating the size of the upcoming reward. The amount of effort required to uncover the reward magnitude cue was systematically varied (0.3 – 1.4s) to examine the relative valuation factoring into the cost and benefit associated with revealing the information across social contexts.
Preliminary results show that revealing the non-contingent information about the upcoming reward value critically depended on the effort required, such that the monkeys seek information more when the cost is low. Monkeys also developed information seeking preferences in line with their underlying social reward sharing preferences, such that a prosocial preference was associated with seeking the information about other-relevant reward value and an antisocial preference was associated with seeking the information about other-irrelevant reward value. Notably, with a prosocial preference, preferentially seeking the information about the donated value over the wasted value was specific to when the effort required was high. These results indicate that curiosity about others is determined by the information cost, suggesting that social curiosity in monkeys is controlled by the tradeoff between cost and benefit of obtaining the information.

Disclosures: J.A. Joiner: None. **S.W.C. Chang:** None.

Poster

542. Motivation and Economic Processes

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Topic: G.02. Motivation

Support: Alfred P. Sloan Foundation

NIMH R00 MH099093

Title: Social information foraging obeys the marginal value theorem in rhesus macaques

Authors: *C. TURRIN[1], N. FAGAN[1], O. DAL MONTE[1], S. W. C. CHANG[2];
[1]Psychology, [2]Psychology and Neurobio., Yale Univ., New Haven, CT

Abstract: Like food, water and other essential resources, animals aggregate in clumped distributions based on specific landscape and biotic features of an environment. It has been demonstrated across a variety of taxa that animals forage for patchily distributed resources according to an energy-maximizing strategy, balancing the tradeoff between the benefits of obtaining energetic gain and the costs of searching. For social species, seeking out conspecifics and effectively attending to and interpreting the social signals of others are critical to reproduction and survival. However, it remains unknown how animals forage for social information. To determine whether social information foraging behavior resembles nonsocial resource foraging behavior, we investigated how rhesus macaques (*Macaca mulatta*) forage for social emotions of conspecific faces. Monkeys completed a virtual social foraging task in which they explored environments containing patches of social information. While foraging, monkeys could explore and exploit specific social information by selecting targets mapped onto emotional valence of conspecific faces that were revealed upon selection. Monkeys could also choose to leave the current patch for a new patch at any time by selecting a travel bar, which indicated randomly varied travel delay length (time to a new patch). Monkeys had a consistent preference for negative-valence targets, selecting them substantially more and earlier within a given environment, suggesting an intrinsically greater informational value of negative-valence images relative to positive-valence images. Looking behavior at facial regions of interest (ROIs: whole face, eyes, mouth) differed significantly for negative- and positive-valence images. Furthermore, between-subject differences in looking time within ROIs reflected differences in social information gathering strategies or social reward valuation by the social status of foragers. We used the Marginal Value Theorem (MVT; Charnov, 1976, Theor Popul Biol), an optimality model that describes the behavior of an animal foraging among patches of resources, to assess whether monkeys foraged for social information using an optimal strategy. Patch residence times during social foraging increased with longer travel delay times, which is consistent with MVT. Our results indicate that social information foraging obeys MVT, suggesting that some of the neural computations used for nonsocial resource foraging (Hayden et al., 2011, Nat Neurosci) may underlie decisions concerning the tradeoff between the benefits of obtaining social information and the search costs.

Disclosures: C. Turrin: None. **N. Fagan:** None. **O. Dal Monte:** None. **S.W.C. Chang:** None.

Poster

542. Motivation and Economic Processes

Location: Halls B-H

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Topic: G.02. Motivation

Support: Wellcome Trust

Title: In economic choices between bundles of goods monkeys satisfy basic assumptions of revealed preference theory

Authors: *A. PASTOR-BERNIER, W. SCHULTZ;
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Abstract: HYPOTHESIS This study assesses trading behavior in non-human primates using formal concepts of revealed preference theory in economics. We investigated how rhesus monkeys handle rewards in a manner that presages the use of bundles in human economic choices. Are their choices compatible with the notion of giving up one good in order to obtain another good? Does this behavior satisfy basic properties of revealed preference theory? GOAL We studied choices between bundles of two juices that allowed the animals to give up some quantity of one in order to gain one unit of the other without a change in overall utility. The bundles contained a common juice (blackcurrant) and one of several other juices such as grape, strawberry, water, blackcurrant juice itself, apple squash, lemon juice, yoghurt, salt (NaCl) solution, and combinations with monosodium glutamate (MSG) and inosine monophosphate (IMP). The different quantities of the two bundle goods combining to the same utility can be graphed as indifference curves, which are didactically a central concept in microeconomics. METHOD Monkeys chose between two bundles by touching specific stimuli on a touch-sensitive monitor. Each bundle contained the same two juices (e.g. blackcurrant and grape) whose quantity was indicated by the height of a bar within a vertical rectangle. One bundle was composed of a fixed quantity of each juice (reference). The other bundle (variable) had a specifically set quantity of grape juice and a quantity of blackcurrant juice that was varied psychophysically to determine choice indifference between the two bundles (50%±5%). Then we increased the quantity of grape juice by one unit (0.1 ml) in the variable bundle and determined psychophysically the quantity of blackcurrant juice that was necessary for regaining choice indifference against the unchanged reference bundle. In this way, we tested how much blackcurrant juice the animal was ready to give up in order to gain one additional unit of grape juice (‘marginal rate of substitution’) without changing overall utility. RESULTS We established at total of 52,500 indifference points in 11 and 4 different bundles in monkeys A and B, respectively (about 3,500 indifference points per bundle type and indifference map per animal). We determined empirically maps of indifference curves with different utility levels and derived the marginal rate of substitution of several juices against blackcurrant juice. Choices made along the indifference curves were internally consistent, transitive and satisfied GARP (Generalized Axiom of Revealed Preferences)

Disclosures: **A. Pastor-Bernier:** None. **W. Schultz:** None.

Poster

542. Motivation and Economic Processes

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Topic: G.02. Motivation

Support: Wellcome Trust 095495

National Institutes of Health Caltech Conte Center

Title: Dynamic integration of reward parameters into economic decision variables by single neurons in the monkey amygdala

Authors: *F. GRABENHORST[1], R. BAEZ-MENDOZA[2], W. SCHULTZ[1];
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Abstract: Recent neurophysiological data suggest a role for the primate amygdala in economic decisions, beyond its known functions in fear and reward. A fundamental process in economic decision-making is the formation of a decision variable, based on the integration of different reward parameters. Here we tested whether individual amygdala neurons extract information about two principal reward parameters—probability and magnitude—from sequential visual cues, and integrate this information into economic decision parameters, including expected reward value and reward (variance) risk. We recorded the activity of 483 amygdala neurons while two monkeys viewed sequential stimuli that signaled separately the probability and magnitude of upcoming liquid rewards. On each trial, probability and magnitude cues were drawn randomly from a set of 15 combinations, thus requiring flexible, dynamic integration. Sequential, non-overlapping cue presentation separated the influence of probability and magnitude information on neuronal responses and allowed us to test their integration over the timecourse of single trials. Behavioral data confirmed accurate reward expectation based on probability and magnitude cues and choice sensitivity to expected value and risk. During sequential presentation of probability and magnitude cues, a significant number of amygdala neurons encoded the cued reward parameter (probability: 105/483 neurons, 22%; magnitude: 125/483 neurons, 26%). Over sequential cue phases, about half of the neurons with initial probability encoding subsequently encoded reward magnitude (and vice versa). Thus, individual neurons processed both probability and magnitude dynamically over time, consistent with potential integration of these reward parameters. As soon as probability and magnitude cues had been shown, a substantial number of neurons (95/483, 20%) encoded either the expected value or risk of the upcoming reward outcome. Crucially, these parameters were not explicitly cued but had to be computed internally based on cued probability and magnitude information. Some of these neurons seemed to explicitly reflect this internal computation, as they showed dynamic

coding transitions that linked initial probability and magnitude signals with subsequent expected value or risk signals. These data suggest that individual amygdala neurons flexibly and dynamically integrate basic reward parameters into economic decision variables.

Disclosures: F. Grabenhorst: None. **R. Baez-Mendoza:** None. **W. Schultz:** None.

Poster

542. Motivation and Economic Processes

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Topic: G.02. Motivation

Support: Wellcome trust

Title: Eliciting values with the Becker De-Groot-Marschak method (BDM) in non-human primates.

Authors: *A. AL-MOHAMMAD, W. SCHULTZ;
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Abstract: Objectives
Current methods of estimating economic (reward) value in animals require repeated choices - thus, values are inferred over the course of many trials. We sought to bring one of experimental economics' most powerful tools of value estimation, providing trial-by-trial value estimates, to research in non-human primates: the Becker De-Groot Marschak method (BDM).
In the BDM, subjects bid for a reward against a computer. If the subject's bid is higher than the computer's then they receive the reward and pay an amount equal to the computer's bid. If the subject's bid is lower than the computer's, then they pay nothing but do not receive the reward. Hence, the BDM is strategically equivalent to a 'second-price' auction, and given few assumptions regarding the subjects' preferences, the optimal strategy is to bid one's true value.
Method
Macaque (rhesus) monkeys were trained in a BDM analogue. They learnt that the area of a bar stimulus on a computer display represented a given volume of juice A (the 'currency' with which to bid), and that a second fractal stimulus represented a given volume of juice B (the reward to bid for).
Monkeys used a joystick to move a red marker across the bar stimulus, its resting location indicating their bid. A second, green, marker appeared to indicate the computer's bid. In the event of a winning bid, an area from the bottom of the bar to the computer's bid was occluded, indicating the volume of 'currency' juice that had been paid - the animal then received the

remaining currency (corresponding to the un-occluded area of the bar), and the reward (indicated by the fractal). In the event of a losing bid, the fractal disappeared (as the reward had not been won), and the animal received all of the 'currency' juice available for that trial, as no 'payment' had been made.
We compared each animal's BDM valuation using an equivalent binary choice task, which estimated subjective values for the various rewards used in the BDM (juice B) in units of the currency used in the BDM (juice A) - thereby checking the validity of values elicited in the BDM.

Results

Data from two animals suggests that the BDM can produce ranking of rewards by order of preference, in both continuous and discrete versions of the task, and with several reward and currency combinations.
Whilst rankings were the same in the BDM and binary choice tasks, absolute values elicited in the two tasks differed. We altered the distribution of computer bids to increase the costs of sub-optimal bidding and observed more consistent bidding following this manipulation. Control tests confirmed that reward value is the main driver of bids in the first monkey, and are currently being tested in the second monkey.

Disclosures: A. Al-Mohammad: None. **W. Schultz:** None.

Poster

542. Motivation and Economic Processes

Location: Halls B-H

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Program#/Poster#: 542.07/HHH36

Topic: G.02. Motivation

Title: Investigating the role of the prefrontal cortex in central amygdala-induced risky decision-making

Authors: *R. TOM[1], M. J. F. ROBINSON[2];
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Abstract: Addiction is characterized by a focused, intense pursuit of one reward above all others, even despite a risk of adverse consequences. The compulsive pursuit of reward has been shown to involve structures in the mesolimbic dopaminergic pathway of the brain. The central amygdala (CeA) plays a role in this pathway, and optogenetic stimulation (ChR2) of this area has previously been shown to assign an increased value to the reward with which it is paired (Robinson et al., 2014).
Our current research shows that rats will continue to pursue CeA stimulation-paired reward, even

when associated with a risk of foot shock. This behavior is thought to come from excessive motivation for disadvantageous reward, and an inability to restrain this motivation. One proposition is that while the CeA may be responsible for the former, the prefrontal cortex (PFC) is critical for the latter, as evidenced by studies showing that drug addiction can cause a reduction in PFC gray matter volume as well as hypoactivity of PFC neurons (Chen et al., 2013). If loss of inhibitory control in the ChR2-CeA rats is in part due to decreased functioning in the PFC, then a restoration of PFC activity should decrease risk-taking behavior (Chen et al., 2013). Here, we investigated this both through amphetamine administration as well as stimulation mediated by designer receptors exclusively activated by designer drugs (DREADDs). Rats are initially trained on an operant task in which they can choose to press either of two levers: one delivering a sucrose pellet, and the other delivering a sucrose pellet and laser stimulation of the CeA. After establishing a >95% preference for the laser-paired reward, conditions are changed such that the lever that previously delivered a sucrose pellet + CeA laser stimulation, is now also associated with a probabilistic risk of foot shock. Animals are then tested with and without stimulation of the PFC (via amphetamine or DREADDs) to demonstrate the impact of PFC stimulation on CeA-driven risky decision-making. These experiments will provide further insight into the role of the CeA in risky decision-making, and the potential moderation of these decisions by the PFC.

References

Chen BT, Yau HJ, Hatch C, Kusumoto-Yoshida I, Cho SL, Hopf FW, Bonci A (2013) Rescuing cocaine-induced prefrontal cortex hypoactivity prevents compulsive cocaine seeking. Nature 496:359-362.

Robinson MJ, Warlow SM, Berridge KC (2014) Optogenetic excitation of central amygdala amplifies and narrows incentive motivation to pursue one reward above another. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:16567-16580.

Disclosures: R. Tom: None. **M.J.F. Robinson:** None.

Poster

542. Motivation and Economic Processes

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Program#/Poster#: 542.08/HHH37

Topic: G.02. Motivation

Title: Primed cues and optogenetic inhibition of the frontal cortex generate shifts in risky decision-making

Authors: *C. FREELAND[1], A. AHUJA[2], F. G. AYRES[2], M. J. F. ROBINSON[3];
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Abstract: Adaptive decision-making involves successfully weighing potential benefits against negative consequences when picking between risky and safe choices. However when the underlying neural systems become skewed to favor risky outcomes, this can lead to unhealthy behaviors and even addiction. Both past memories and internal neural mechanisms affect our ability to make rational decisions in the moment. Previous research has shown that human participants primed with cues previously paired with large risky wins are more likely to choose the riskier option. Imaging studies have also shown that the frontal cortex, particularly the anterior insular cortex (AIC) and orbitofrontal cortex (OFC), are implicated in risky decision-making. Yet little is known about how decisions are made moment-to-moment especially in the face of uncertainty and risk. In this study, we examined how priming with either win and loss cues and activity in specific brain regions influence risky decision-making. First, rats were trained on an operant task that required them to choose between a risky option that offered a large but uncertain (50%) reward and a safe option that offered a small but certain (100%) reward. Each possible outcome (small safe win, large risky win and risky loss) was accompanied by one of three distinct auditory cues. After training, rats completed a testing phase in which the auditory cues preceded (primed) each choice trial but were not predictive of the reward outcome. We found that rats chose the risky option significantly more often following primed win cues and less often following loss cues. Additionally, female rats appeared to be more sensitive to primed cues than males. Next, to pinpoint the role of the AIC and OFC in the decision-making process, we utilized the temporal sensitivity afforded by optogenetics to inhibit these structures at brief specific time points. Following the same operant training regimen, optogenetic inhibition was administered during the task in three possible ways (pre-choice, post-choice following risky win trials and post-choice following risky loss trials) at each site on separate testing days. Our findings suggest that inhibiting the AIC either prior to choice, or post-choice on risky win trials, significantly shifted risky preference. This suggests that the AIC may be involved in decision-making ‘in the moment’ by tracking win outcomes. Together these results begin to uncover the interplay of environmental cues and neural systems that affect our choices under conditions of uncertainty and risk.

Disclosures: C. Freeland: None. **A. Ahuja:** None. **F.G. Ayres:** None. **M.J.F. Robinson:** None.

Poster

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Topic: G.02. Motivation

Support: DA007267

DA015188

MH63649

Title: Optogenetic modulation of desire and dread motivations in the nucleus accumbens shell

Authors: *S. L. COLE, N. A. MOSTOVOI, K. C. BERRIDGE;
Univ. of Michigan, Ann Arbor, MI

Abstract: The nucleus accumbens (NAc) shell is famous for reward, but also can generate fearful behaviors following an anatomical 'desire-dread' rostrocaudal pattern. This is revealed by microinjections that relatively inhibit NAc neurons (e.g., DNQX). Here, we tested whether neuronal inhibition is crucial, by using optogenetic *depolarization* to combat DNQX-inhibition at the same NAc site. Our results suggest this is possible: channelrhodopsin (ChR2) depolarization of NAc neurons reduced eating behavior elicited by DNQX microinjection at the same site in rostral shell. Further, laser ChR2 depolarization had to overlap with microinjection site to reduce DNQX-induced eating. Finally, we are testing whether optogenetic inhibition via halorhodopsin is sufficient by itself in rostral shell to induce eating behavior. Preliminary results suggest the answer is yes: in a laser stimulation-bound fashion, laser *hyperpolarization* induces intense bouts of eating. Together, these findings demonstrate that NAc hyperpolarization is both necessary for DNQX-mediated motivation and sufficient to produce intense motivated behavior.

Disclosures: S.L. Cole: None. **N.A. Mostovoi:** None. **K.C. Berridge:** None.

Poster

542. Motivation and Economic Processes

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Topic: G.02. Motivation

Support: NIH T32 DC00011

Title: Disgust and brain areas activated by pharmacologic inhibition of the posterior ventral pallidum

Authors: *K. URSTADT, H. A. KHAN, N. M. RABAH, K. C. BERRIDGE;
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Abstract: Pathologically excessive disgust can induce negative affect and emotional distress in certain psychiatric disorders, driving research to understand the neural substrates of disgust. The posterior lateral ventral pallidum (plVP) is the only brain area wherein lesions produce pathological disgust, thereby converting normal liking of sucrose to disgusted gapes. However, few other disgust generators in the brain have been identified; these may be disinhibited by plVP inhibition. We highlighted activation of such disgust generator candidates via c-fos immunohistochemistry. We also observed how plVP inhibition alters multiple behaviors. A muscimol and baclofen cocktail (MB) or vehicle was injected directly into the plVP of adult male and female Sprague-Dawley rats. MB treatment strongly increased c-fos expression in posterior accumbens shell, lateral hypothalamus, supramammilary nucleus, substantia nigra, parabrachial nucleus, and paraventricular thalamus. MB treatment also induced gaping, flailing, and hyperkinetic treading responses to oral sucrose solution, suppression of palatable M&M intake, increased social avoidance, and increased bedding treading and head burying behaviors. These data suggest that the plVP regulates an assortment of disgust and avoidance responses, and does so through multiple downstream brain regions that could serve as generators of disgust. Future studies will probe the plVP further with inhibitory DREADD and optogenetic techniques.

Disclosures: K. Urstadt: None. **H.A. Khan:** None. **N.M. Rabah:** None. **K.C. Berridge:** None.

Poster

542. Motivation and Economic Processes

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Topic: G.02. Motivation

Support: NIH Grant DA015188

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NIH Grant T32 DC00011

Title: Central amygdala controls choice and amplifies 'wanting' for sucrose and cocaine without altering 'liking'

Authors: *S. M. WARLOW[1], D. C. CASTRO[2], E. E. NAFFZIGER[1], K. C. BERRIDGE[1]; [1]Psychology, Univ. of Michigan, Ann Arbor, MI; [2]Anesthesiol., Washington Univ. in St. Louis, St. Louis, MO

Abstract: We have previously found that optogenetic stimulation of central amygdala (CeA) but not basolateral amygdala can powerfully bias a rat's choice and increase their motivation for one sucrose reward over another. However, it is unknown how CeA stimulation is generating such a strong bias. One possibility is that the outcome of sucrose is being enhanced. In other words, CeA stimulation is making the sucrose more 'liked'. We tested this possibility by using a taste reactivity test, which assesses the hedonic impact of various tests. We infused a 1% sucrose solution into the mouths of rats via intraoral cannulas while also delivering optogenetic CeA stimulation or not (baseline). We observed no changes in positive (or negative) affective orofacial reactions in response to sucrose in the presence of laser stimulation. This indicates that CeA stimulation was not enhancing incentive motivation by making the sucrose more 'liked', and instead selectively making the sucrose more 'wanted', or desired in the same rats. Further, when pairing CeA stimulation with earning one cocaine infusion (0.3mg/kg/infusion) vs. an identical infusion not paired with laser, rats intensely bias their choice and display increased motivation for CeA-paired cocaine. We additionally observed for the first time numerous consummatory bite, nibble, and sniff actions directed only at the CS nose port delivering CeA stimulation with cocaine. Since CeA stimulation is capable of biasing and enhancing incentive motivation, we next asked whether CeA stimulation could control which reward gets 'wanted' most. We arbitrarily paired CeA stimulation with earning either a sugar pellet or a cocaine infusion (0.3mg/kg/infusion), and gave rats a choice between the two rewards in the same session. We are also exploring the role of CeA subdivisions in enhancing and biasing incentive motivation. We selectively stimulated either medial or lateral portions of CeA during an operant task where rats chose between a laser-paired sucrose and an identical sucrose not paired with laser. These experiments have the potential to highlight specific central amygdala circuitry capable of generating focused desire for reward in an addictive-type fashion.

Disclosures: S.M. Warlow: None. **D.C. Castro:** None. **E.E. Naffziger:** None. **K.C. Berridge:** None.

Poster

542. Motivation and Economic Processes

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Topic: G.02. Motivation

Support: NIH Grant DA015188

Title: Optogenetic stimulation of the medial amygdala biases choice and enhances motivation for sucrose

Authors: *E. E. NAFFZIGER, S. M. WARLOW, K. C. BERRIDGE;
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: It has previously been shown that optogenetic stimulation of the central nucleus of the amygdala (CeA) can powerfully bias choice and enhance motivation for the delivery of a laser-paired sucrose pellet over delivery of an identical sucrose pellet. These findings were confined to the CeA, as similar photostimulation of the basolateral amygdala failed to elicit a bias for the sucrose pellet paired with laser stimulation. However, whether the medial nucleus of the amygdala (MeA), which shares several neuroanatomical similarities with the CeA, would similarly alter choice is unknown. We report novel data indicating that the MeA, comparable to the CeA, is capable of biasing choice and enhancing motivation for a sucrose reward that has been paired with MeA photostimulation. Preliminary evidence suggests that photoexcitation of the MeA by itself may be sufficient to establish self-stimulation behavior in at least some individuals. Together this data indicates that while the MeA is also capable of focusing choice and enhancing motivation for a sucrose reward, it may be doing so via somewhat different psychological mechanisms than the CeA.

Disclosures: E.E. Naffziger: None. **S.M. Warlow:** None. **K.C. Berridge:** None.

Poster

542. Motivation and Economic Processes

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Topic: G.02. Motivation

Support: CIHR

NSERC

Title: Optogenetic stimulation of the median raphe nucleus produces anxiety-like behavior

Authors: *A. R. ABELA[1], C. J. BROWNE[1,2], A. D. LÊ[1], P. J. FLETCHER[1];
[1]Psychology, CAMH, Toronto, ON, Canada; [2]Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: There is a large body of evidence indicating that serotonin (5-hydroxytryptamine; 5-HT) neurons originating in the midbrain raphe nuclei contribute to the regulation of stress and anxiety. In laboratory animals, anxiety-like behavior is assessed using tests of approach-avoidance conflict in an innately fear-provoking context (e.g., a rodent's preference for a safe, closed space over a vulnerable, open space). Pharmacological and genetic manipulations that impair the action of the serotonin transporter in rodents (thereby enhancing 5-HT activity) induce avoidance of open spaces (Olivier et al., Neuroscience, 2008; Drapier et al., Behav Brain Res, 2007). A similar anxiogenic response is produced by lesions of the median raphe nucleus (MRN)—a major source of 5-HT output to the rest of the brain (Andrade and Graeff, Pharm, Biochem and Behav, 2001). However, pharmacological manipulations often produce non-selective receptor activity at other targets, or in the case of genetic knock-out animals, there is the possibility of compensatory mechanisms that occur during development. We examined whether optogenetic stimulation of 5-HT neurons originating in the MRN modulated the expression of anxiety-like behavior in three approach-avoidance contexts: 1) the elevated-plus maze, 2) novelty-suppressed feeding, and 3) the open-field test. The optogenetic construct was created through expression of channel rhodopsin (ChR2) under the control of a serotonergic cre-driver, using a cross-breeding procedure of two mouse lines. Ai32 mice, which express ChR2 in response to cre recombinase through excision of an upstream floxed-STOP cassette, were crossed with ePet-cre mice producing ChR2 expression restricted to 5-HT neurons. A chronically implanted optical fiber enabled delivery of 10 ms pulses of 10 mW blue light to the MRN. Optogenetic stimulation of the MRN at 4 Hz for 3 min caused animals to avoid the open arms of the elevated-plus maze, a behavior that was not observed in other periods absent of stimulation. Continuous optogenetic stimulation increased the latency at which animals investigated and consumed food in a novel context, but not when the context was familiar. In the open field test, optogenetic stimulation of the MRN had no effect on the amount of time spent in the centre, the distance travelled or the average speed of the animals. These results demonstrate that 5-HT output from the MRN rapidly regulates the expression of anxiety-like behaviors, in contexts that innately illicit fear and approach-avoidance conflict. Future studies will examine how 5-HT output from the MRN interacts with specific downstream structures to produce these changes in behavior.

Disclosures: **A.R. Abela:** None. **C.J. Browne:** None. **A.D. Lê:** None. **P.J. Fletcher:** None.

Poster

542. Motivation and Economic Processes

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Topic: G.02. Motivation

Support: CIHR

NSERC

Title: Effects of pharmacological, genetic, and optogenetic enhancement of serotonin activity on responding for a primary reinforcer

Authors: *C. BROWNE[1,3], X. JI[3], P. J. FLETCHER[1,2,3];
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Abstract: Serotonin (5-hydroxytryptamine; 5-HT) neurons originating in the midbrain raphe nuclei innervate virtually the entire brain and have been implicated in the control of many behavioural processes. A large body of evidence suggests that reward-related behaviours are particularly sensitive to changes in 5-HT activity. Manipulations that increase 5-HT typically reduce behaviours such as operant responding for primary reinforcers, while these behaviours are potentiated following decreases in 5-HT activity. While the mechanism of this serotonergic inhibition of motivated behaviour is unknown, it is hypothesized to involve 5-HT neurons in the dorsal raphe nucleus (DRN) that densely innervate brain regions involved in reward processing. In these experiments, we first examined the effects of acute or chronically elevated whole-brain 5-HT on lever pressing for the primary reinforcer saccharin (0.2%, 0.1 ml) on an RR4 schedule of reinforcement in mice. Acute blockade of the serotonin transporter (SERT) with 10 mg/kg of the selective 5-HT reuptake inhibitor citalopram significantly reduced responding for saccharin. Similarly, mice with a constitutive genetic deletion of the SERT (SERT-KO), which chronically elevates extracellular 5-HT, made fewer responses for saccharin compared to wild-type littermates. Next, we examined whether optogenetic activation of DRN 5-HT neurons could recapitulate the inhibitory effects elevated 5-HT on responding for saccharin. To generate this optogentic construct, Ai32 mice which express ChR2 through cre-mediated excision of an upstream floxed-STOP cassette were crossed with the serotonergic cre-driver line ePet-cre. A chronically implanted optical fiber enabled delivery of 10 ms pulses of 10 mW blue light to the DRN. Photostimulation of the DRN had no effect on responding for saccharin at a range of different frequencies (1, 5, 10, 20 Hz). However, when 10 Hz DRN stimulation was coupled with a subthreshold dose of 5 mg/kg citalopram, a significant reduction in responding for saccharin compared to citalopram-treated control mice was observed. Temporal analysis of the test sessions found that this effect was largely due to the combination of citalopram and DRN

photostimulation changing the overall pattern of behaviour. Together, these results implicate DRN 5-HT output in the control of incentive motivation, but suggest that these effects may be dependent upon an interaction between SERT function and levels of extracellular 5-HT, as opposed to frequency modulation of DRN 5-HT neurons.

Disclosures: **C. Browne:** None. **X. Ji:** None. **P.J. Fletcher:** None.

Poster

542. Motivation and Economic Processes

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 542.15/III4

Topic: G.02. Motivation

Support: CIHR

Title: Serotonin exerts tonic inhibitory control over mesolimbic dopamine release and dopamine-dependent behaviour through the 5-HT_{2C} receptor.

Authors: ***C. HARVEY-LEWIS**[1], C. J. BROWNE[1,2], T. JI[1], G. A. HIGGINS[3,4], P. J. FLETCHER[1,2,3];
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Abstract: The serotonin 2C (5-HT_{2C}) receptor system is hypothesized to influence reward-related processes through modulatory control of the mesolimbic dopamine (DA) system. Previous studies have shown that 5-HT_{2C} receptor agonists decrease mesolimbic DA activity and dopamine-dependent behaviors. Similar studies have shown that 5-HT_{2C} antagonists can increase mesolimbic DA release, but it is unknown whether this has any direct influence on behaviour. Here we confirm the effects of the 5-HT_{2C} antagonist, SB242084, on nucleus accumbens DA release and investigate the effect of SB242084 on two dopaminergic-dependent behaviors - responding for a conditioned reinforcer (CRf) and locomotor activity. For microdialysis experiments, one group of mice received guide cannula implants above the nucleus accumbens, through which a dialysis probe was inserted into the accumbens one week later. In chronically anesthetized mice, dialysate samples were collected prior to, and following, an IP injection of 0.5mg/kg SB242084 or vehicle and analyzed online using high performance liquid chromatography. Two additional groups of mice were used for behavioral experiments. One group of mice received 5 days of habituation to locomotor chambers. Following training, animals were treated with SB242084(0-1mg/kg) 20min prior to locomotor testing. The other group of

mice was given 14d of Pavlovian conditioning during which a CS was paired with the delivery of 0.1ml 0.2% saccharin. Subsequently, mice were given the opportunity to lever press for the presentation of the CS (now a CRf) without saccharin. Animals were given SB242084 (0-1mg/kg) 20-min prior to tests on this task. In a second series of tests, this group of animals received the broad-spectrum DA receptor antagonist flupenthixol (0.25mg/kg) prior to SB242084 (0.5mg/kg) and were then allowed to respond for a CRf. We found that SB242084 caused approximately a 20% increase in nucleus accumbens dopamine concentration when compared to preinjection baseline. Behaviorally, SB242084 caused significant increases locomotor activity and CRf-directed responding. In addition, treatment with a silent dose of flupenthixol blocked the SB242084 induced increases in responding for a CRf. Together, these results provide support the ability of serotonin to tonically suppress mesolimbic DA activity and DA-dependent motivated behavior through the 5-HT_{2C} receptor.

Disclosures: C. Harvey-Lewis: None. **C.J. Browne:** None. **T. Ji:** None. **G.A. Higgins:** None. **P.J. Fletcher:** None.

Poster

542. Motivation and Economic Processes

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 542.16/III5

Topic: G.02. Motivation

Support: NIH Grant MH106972

NIH Grant T32 DA024635-07

Title: Contribution of nucleus accumbens core cholinergic interneurons to cue-motivated behavior

Authors: *A. L. COLLINS[1], T. J. AITKEN[1], S. B. OSTLUND[2], K. WASSUM[1,2];
[1]UCLA, Los Angeles, CA; [2]UCI, Irvine, CA

Abstract: Environmental reward-predictive stimuli provide a major source of motivation for reward-seeking behaviors. Considerable evidence has implicated the nucleus accumbens core (NAc) and dopamine signaling therein in cue-motivated behavior, but relatively little is known about how other striatal neuromodulatory systems contribute. Our recent data suggest that the NAc cholinergic system may also critically modulate cue-motivated behavior, possibly through terminally modulating dopamine release. Cholinergic interneurons (CINs) provide the main source of acetylcholine to the striatum, and their activity has been shown to be elevated in

situations that discourage vigorous reward seeking and depressed in response to reward-predictive cues, which encourage reward seeking. These data suggest that NAc CIN activity may gate the expression of cue-motivated behavior, having a suppressive effect when elevated and a permissive effect when depressed. To test this, we examined the causal role of CIN activity in cue-motivated behavior using the Pavlovian-to-instrumental transfer (PIT) task designed to assess the motivating influence of a reward-predictive cue (CS+) over an independently-trained instrumental action. CIN activity was optically stimulated concurrent with CS+ presentation and this was compared to the influence of CIN stimulation unpaired with CS+ presentation. Findings that CIN stimulation blunts CS+-invigorated reward seeking will provide causal support the above hypothesis.

Disclosures: **A.L. Collins:** None. **T.J. Aitken:** None. **S.B. Ostlund:** None. **K. Wassum:** None.

Poster

542. Motivation and Economic Processes

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 542.17/III6

Topic: G.02. Motivation

Support: NIH Grant MH106972

NIH Grant T32 DA024635-07

UCLA HH Undergraduate Research Program

Title: Contribution of nucleus accumbens core cholinergic interneuron tonic activity to cue-motivated behavior

Authors: ***T. AITKEN**[1], A. L. COLLINS[1], S. B. OSTLUND[2], K. M. WASSUM[1,2]; [1]Psychology, UCLA, Los Angeles, CA; [2]UCI, Irvine, CA

Abstract: Environmental reward-predictive stimuli provide a major source of motivation for reward-seeking behaviors. Considerable evidence has implicated the nucleus accumbens core (NAc) and dopamine signaling therein in cue-motivated behavior, but relatively little is known about how other neuromodulatory systems contribute. Our recent data suggest that the NAc cholinergic system may also critically modulate cue-motivated behavior, possibly through terminally modulating dopamine release. Cholinergic interneurons (CINs) provide the primary source of acetylcholine acting at these receptors and activity of these neurons has been shown to correlate with the presentation of reward-predictive stimuli, motivated behavior, and satiety. Therefore, here we examined the function of these NAc CINs in cue-motivated behavior using a

Pavlovian-to-Instrumental transfer (PIT) task designed to assess the motivating influence of a food-predictive cue over an independently-trained, instrumental, food-seeking action. Inhibitory DREADDs were used to inactivate NAc CINs during a PIT test while rats were either hungry or sated. Preliminary data indicate that attenuation of NAc CIN activity augmented cue-induced invigoration of reward seeking, suggesting that NAc CIN activity may gate the expression of cue-motivated behavior. Follow-up tests are exploring additional functions of the NAc CINs in motivated behavior and are examining a possible dopaminergic mechanism for this effect.

Disclosures: T. Aitken: None. **A.L. Collins:** None. **S.B. Ostlund:** None. **K.M. Wassum:** None.

Poster

542. Motivation and Economic Processes

Location: Halls B-H

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Program#/Poster#: 542.18/III7

Topic: G.02. Motivation

Support: NIH Grant R01DK106188

Brain and Behavior Foundation Grant N018940

Title: Estrous cycle phase modulates cue-triggered motivation for food in obesity-prone but not in obesity-resistant rats

Authors: *Y. ALONSO CARABALLO[1], C. R. FERRARIO[2];
[1]Neurosci. Grad. Program, [2]Dept. of Pharmacol., Univ. of Michigan, Ann Arbor, MI

Abstract: In females, naturally occurring alterations in estradiol influence food intake and food preference. However, how the cycle alters motivation for food is poorly understood. Furthermore, weight gain disrupts the reproductive cycle and is associated with enhanced motivation for and consumption of sugary, fatty foods, particularly in obesity-susceptible individuals. Here, we determined how food intake and motivation for food vary across the estrous cycle in female obesity-prone vs. obesity-resistant rats. Rats were housed in reverse light/dark conditions, weight was measured daily, and vaginal lavage was used to determine estrous cycle phase. Home cage food intake was measured for 13 days in singly housed females (Lab Diet 5001). Changes in motivation across the cycle were examined in a separate set of pair housed females using Pavlovian conditioned approach procedures. Specifically, rats were trained to associate one auditory cue (CS+; 2 min) with sucrose delivery (45 mg pellets; TestDiet) whereas a second cue (CS-; 2 min) was never paired with sucrose (5 sessions, 1 session/d). Rats

were then repeatedly tested for conditioned approach, a measure of motivation, at each stage of the cycle. The test consisted of 4 presentations of the CS+ and CS- in the absence of sucrose. We found that daily home cage chow consumption and body weight were greater in female obesity-prone vs. obesity-resistant rats. Estrous cyclicity was similar between groups. In addition, home cage food intake during estrus decreased in all rats. When motivation was examined, we found that the estrous cycle modulates conditioned approach behavior in obesity-prone but not obesity-resistant rats. Specifically, the magnitude of approach to the CS+ was reduced in proestrus and estrus compared to diestrus in obesity-prone rats, but remained stable across the cycle in obesity-resistant rats. This was somewhat surprising given that home cage food intake and weight decreased during estrus in both groups. Furthermore, when comparisons were made between obesity-prone and -resistant groups, we found that the magnitude of approach behavior during the CS+ tended to be higher in obesity-prone vs obesity-resistant rats. This difference was most pronounced during proestrus and metestrus, when motivation was highest in obesity-prone rats. In rats and humans, enhanced motivational responses to stimuli paired with food predict subsequent weight gain. The data here suggest that motivation for and consumption of food is enhanced in female obesity-prone rats, and that the ability of naturally occurring changes in hormonal levels to modulate motivation is also enhanced relative to obesity-resistant rats.

Disclosures: Y. Alonso Caraballo: None. **C.R. Ferrario:** None.

Poster

542. Motivation and Economic Processes

Location: Halls B-H

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Program#/Poster#: 542.19/DP07 (Dynamic Poster)

Topic: G.02. Motivation

Support: NIH R01DK106188

NIH 1F31DK111194-01

Title: Calcium-permeable AMPA receptors in the nucleus accumbens mediate cue-triggered food-seeking in obesity-prone rats.

Authors: *R. C. DERMAN[1], C. R. FERRARIO[2];
[2]Pharmacol., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Studies in humans and rats show that obesity-susceptible individuals are more sensitive to the motivational impact of stimuli associated with food (i.e., conditioned stimuli). Using Pavlovian-to-instrumental transfer (PIT), a classic measure of the motivational strength of

conditioned stimuli (CS), we found that selectively bred obesity-prone (OP) rats exhibit enhanced PIT relative to obesity-resistant (OR) rats. The expression of PIT relies on activation of the NAc and AMPARs provide the main source of excitation to the NAc. Here, we measured NAc AMPAR subunit expression after initial training and the effect of intra-NAc AMPAR blockade on the expression of PIT. Male OP and OR rats learned to press a lever to earn food pellets (Bioserv). Next, in Pavlovian training, they learned to discriminate between an auditory CS+ that was always paired with food and a CS- that was never paired with food. A set of untrained control rats were handled daily. After training or control handling, NAc tissue was collected and cross-linked with BS^3 enabling the comparison of surface vs. intracellular proteins. Thus, these data provide a "snapshot" of AMPAR expression corresponding to the time when PIT testing would normally occur. For intra-NAc infusion studies, rats were trained as described above. Following training they were surgically implanted with bilateral guide cannulae aimed at the NAc core. Rats were given 2 additional instrumental and Pavlovian training sessions before PIT testing. Rats were tested repeatedly after intra-NAc infusion of vehicle, Naspm (20ug/0.5ul) a selective antagonist of calcium-permeable AMPARs (CP-AMPAR), or the general AMPAR antagonist CNQX (0.5ug/ul), in a counter-balanced design. During PIT testing, pellet delivery was omitted, levers were continuously available, and each CS was presented 4 times. We found that training increased NAc surface vs intracellular GluA1 expression in OP, but not OR rats. No changes were found in GluA2 expression in either group. An increase in GluA1 without changes in GluA2 suggests an increase in CP-AMPARs, as these receptors lack the GluA2 subunit. During PIT testing, we found that CNQX produced a general reduction in lever pressing in OR rats, but did not disrupt the selective invigoration of lever pressing by the CS+ (i.e., PIT), while CNQX had no effect in OP rats. In contrast, Naspm eliminated PIT in OP rats, but had only modestly effects in OR rats. Together, our data show that training selectively increases NAc CP-AMPARs in OP but not OR rats and that NAc core CP-AMPARs selectively mediate the expression of PIT, a form of cue-triggered motivation. Finally, effects of CP-AMPAR blockade were more pronounced in OP rats.

Disclosures: R.C. Derman: None. **C.R. Ferrario:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 543.01/III8

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant NINDS-NS078127

Klingenstein Fund

Simons Collaboration on the Global Brain

Title: Representation of contextual information in cortico-basal ganglia circuits during motor timing

Authors: *E. HOSSEINI[1], J. WANG[2], M. JAZAYERI[1,2];
[1]Brain and Cognitive Sci., [2]McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: Humans and animals can flexibly adjust their motor plans based on behavioral context. The cortico-basal ganglia circuits have been implicated in controlling context-dependent motor planning. However, the representation of behavioral context across these circuits is not well understood. To address this question, we recorded neural activity from multiple nodes of the circuit including the dorsal medial frontal cortex (dMFC), caudate (Cd) and central/motor thalamus (Thal) in rhesus monkeys trained to produce time intervals in a context-dependent manner. The behavioral task, which we refer to as the Cue-Set-Go (CSG) task, consists of four randomly interleaved trial types in which the animal produces either a "Short" (800 ms) or a "Long" (1500 ms) interval by either a saccade or a manual button press (2 Intervals x 2 Effectors). Each trial begins with the "Cue" period when the animal fixates on a central spot and places a hand on a button. The color and shape of the fixation point provide information about the Interval and Effector, respectively. After a variable delay, a "Set" flash around the fixation point cues the animal to initiate timing. The animal receives reward for initiating the movement with the correct effector and producing an interval that is close to the desired interval. The reinforcement schedule is adjusted throughout the session so that the animal receives reward on approximately half of the trials, and the magnitude of the reward increases with accuracy. Animals were able to perform the task and switch between contexts on a trial-by-trial basis.
We focused our analysis on the representation of the behavioral context. Our initial analysis revealed idiosyncratic selectivity patterns at the level of single neurons in all three areas with no discernible tuning for Effector or Interval. However, there were overall biases in the representation of Interval and/or Effector at the population level that were similar across areas, despite the diverse selectivity patterns of individual neurons. A hierarchical clustering analysis at the population level provided further evidence for the similarity of context representation across areas. Using dimensionality reduction techniques, we found that the two contextual axes (Interval and Effector) were represented in nearly orthogonal subspaces in the caudate, but not in other areas. This observation suggests that the caudate might play a unique role in maximizing the distance between possibly confusable contexts.

Disclosures: **E. Hosseini:** None. **J. Wang:** None. **M. Jazayeri:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

Location: Halls B-H

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Program#/Poster#: 543.02/III9

Topic: H.01. Animal Cognition and Behavior

Support: NINDS-NS078127

Klingenstein Fund

Simons collaboration on the Global Brain

Title: Scalar dynamics in neural activity during timing

Authors: *J. WANG, E. HOSSEINI, M. JAZAYERI;
McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: Human can produce different time intervals in a range of behaviors such as speech production, playing music and sports. Converging evidence suggests an important role for the cortico-basal ganglia circuits in motor timing. However, the coding principles by which these circuits support timing behavior is not known. To address this question, we performed electrophysiology experiments in monkeys trained to perform a motor timing tasks, which we refer to as the Cue-Set-Go (CSG) task. The CSG task consists of four randomly interleaved trial types in which the animal produces either a “Short” (800 ms) or a “Long” (1500 ms) interval by either a saccade or a button press. To initiate a trial, the animal is required to fixate a central spot and place a hand on a button. After a variable delay, a “Set” flash cues the animal to start timing. The animal has to initiate a motor response (Go) around the desired interval with the correct effector to receive reward. Animals were able to switch between Effector and Interval on a trial-by-trial basis. Average production intervals were close to the desired intervals and the variability for both effectors scaled with the interval duration (i.e., scalar variability).
We recorded neural activity in multiple nodes of the cortico-basal ganglia circuits including the dorsomedial frontal cortex (dMFC), the putative target of dMFC in the caudate, and the putative target of basal ganglia in the central thalamus. We analyzed neural activity associated with the production interval between Set and Go. In all three areas, individual neurons had heterogeneous responses and were strongly modulated by elapsed time. Remarkably, the response profiles of many neurons were similar after compressing or stretching the time axis to match the produced interval. We refer to this property as scalar dynamics. Dimensionality reduction of response profiles provided additional evidence for scalar dynamics at the population level, in particular in dMFC and the caudate. The scalar dynamics might be the key factor driving the scalar variability in timing behavior. Moreover, the ability to scale the neural responses on a trial-by-trial basis

puts important constraints on the architecture and mechanisms of cortico-basal ganglia circuits involved in timing.

Disclosures: J. Wang: None. **E. Hosseini:** None. **M. Jazayeri:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 543.03/III10

Topic: H.01. Animal Cognition and Behavior

Support: KIST Grant 2E26640

Title: Amount of training affects CS-evoked neural activation pattern: Implication for a shift in nature of associatively-activated event representation over the course of conditioning

Authors: *H.-Y. KOH[1,2], H.-J. KIM[1,2];
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Abstract: In associative learning, a conditioned stimulus (CS) comes to activate an internal representation of the unconditioned stimulus (US) with which it is paired, and this representation can serve for acquisition of new learning about US. CS (odor)-evoked representation of a US (sugar) can substitute for the actual US itself in the acquisition of an aversion to that US, which is called representation-mediated taste aversion (RMTA). In our previous study using mice, we have shown that RMTA occurs only transiently in the early stage of the initial CS-US conditioning, and CS-nausea pairing can no longer establish an aversion to the US later with extended initial conditioning. It is proposed that the nature of CS-evoked US representation changes over the course of training, in that CS-evoked US representation contains the sensory component available for RMTA learning with a minimal initial training, but no longer does with an extended initial training. Here, we report an evidence for this change of nature of CS-evoked US representation by showing differential patterns of CS-evoked neural activation in brain regions (e.g. insular cortex, IC; nucleus accumbens, NAcc, etc.) using c-Fos immunohistochemistry. In the minimal training condition, rewarded odor (+Odor) induced significantly higher level of c-Fos expression than unrewarded odor (-Odor) in IC, which includes gustatory cortex, and also in NAcc, which is involved in motivation processing. In the extended training condition, +Odor induced significantly higher level of c-Fos expression than -Odor in NAcc, but not in IC. These results suggest that CS-evoked US representation contains both the perceptual and motivational components with a minimal training, and becomes less

perceptual with an extended training. This study would contribute greatly to better understanding of associatively-activated event representation in the field of learning theory.

Disclosures: H. Koh: None. **H. Kim:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

Location: Halls B-H

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Program#/Poster#: 543.04/III11

Topic: H.01. Animal Cognition and Behavior

Support: NIH, NIDA 5SC1DA034995

Title: Decreased excitability of dorsomedial and dorsolateral striatum (by NBQX) undermines normal goal-directed decision making, but not interval timing, in a reward devaluation peak timing task

Authors: *R. I. CAAMANO-TUBIO, N. BALISOK, A. R. DELAMATER;
Brooklyn Col., Brooklyn, NY

Abstract: Prior research implicates the dorsal striatum in interval-timing. The dorsomedial (DMS) and dorsolateral (DLS) striatum have also been shown to play a role in goal-directed and habitual behavior, respectively. The present study examined the role of DMS and DLS in the expression of goal-directed responding on a peak interval timing task. Rats were trained to lever press for qualitatively different food pellet rewards in the presence of different discriminative stimuli (60 s tone, 60 s houselight). A single lever press 20 s after stimulus onset produced a sweet pellet in the presence of one of these stimuli, but a non-sweetened grain pellet in the presence of the other stimulus. Training continued on this task until responding on non-reinforced probe trials revealed a temporally organized pattern of responding across the trial with response rate peaking at approximately 20 s (the time of anticipated reward). Rats then received selective reward devaluation training in which one of the reinforcing outcomes was presented non-contingently (in the absence of the lever or stimuli) in sessions that ended in an injection of the emetic LiCl. The other reinforcing outcome was equally exposed on other sessions, but not paired with LiCl injection. Finally, rats were tested under extinction conditions for lever press responding in the presence of the two discriminative stimuli (one of which now signaled the devalued outcome). Prior to test sessions, two different groups of rats received either intra-DMS or DLS infusions. Each rat received a bilateral infusion of AMPA-receptor antagonist NBQX (0.1 ug delivered in 0.3 ul) and saline in a counterbalance order. We observed that saline-infused rats displayed temporally organized responding in the presence of the stimuli, with responding

peaking shortly after the anticipated time of food delivery; however, responding was lower in the presence of the stimulus signaling the devalued food reward. NBQX administered to either the DMS or DLS disrupted goal-oriented responding, but had minimal impact on the temporal organization of responding across the trial. In particular, NBQX in the DMS eliminated the difference in responding in the presence of the stimulus signaling the valued and devalued rewards. NBQX in the DLS, paradoxically, increased responding in the presence of the stimulus signaling the devalued reward relative to the other stimulus. We observed a similar pattern of results for conditioned magazine approach. These results do not support a significant role for the dorsal striatum in the expression of interval timing, but they do confirm prior results suggesting that it plays a role in goal-directed responding.

Disclosures: R.I. Caamano-Tubio: None. **N. Balisok:** None. **A.R. Delamater:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC graduate fellowship (CGS-D to JG)

Discovery Grant (to MvdM)

Title: Neural coding for distinct sets of reward-predictive cues in the rat ventral striatum

Authors: *J. GMAZ, J. E. CARMICHAEL, M. A. A. VAN DER MEER;
Dartmouth Col., Hanover, NH

Abstract: Background: Adaptive behavior requires learning associations between rewards and the stimuli that predict them, and using this information to guide decisions. These associations are encoded by neurons in the ventral striatum (vStr), which change their firing after presentation of reward-predictive cues in a manner that predicts the vigor and direction of subsequent actions. Natural environments typically contain multiple reward-predictive stimuli, which may span multiple sensory modalities and/or distinct anatomical inputs to the vStr. Moreover, the informativeness of these cues may be context-dependent. How do the currently relevant cues gain control of a final common motivational path? In particular, does the same population of neurons in the vStr encode reward regardless of which cue predicted it, or are there dedicated populations of reward-predictive neurons for each cue?
Behavioral task: To address this issue, we recorded the activity of multiple single neurons in the

vStr as rats performed a task that switches between different sets of reward-predictive cues. Specifically, we trained rats (n = 5) to run on a square track containing four possible reward locations. The presence or absence of sucrose water reward at each upcoming site was signaled by the identity of either a visual or auditory cue, trained in separate blocks (visual cue block: C+ ◊ reward, C- ◊ no reward; auditory cue block: A+ ◊ reward, A- ◊ no reward). After rats discriminated behaviorally between the cues, they were implanted with multielectrode arrays in the vStr, and we recorded from single units during performance of the task.
Neural data: We sought to determine first, what aspects of vStr activity discriminated between the rewarded cues C+ and A+, and second, how such discrimination related to encoding of reward. The principal components of ensemble neural activity around the time of cue presentation were broadly similar during the visual and auditory cues; nevertheless, a substantial proportion of cue-responsive neurons (~30%) distinguished between C+ and A+. A partially overlapping set of neurons distinguished between rewarded and unrewarded cues (~20%). Finally, a subset of neurons exhibited location-specific firing patterns, even though the task contained no such associations.
Conclusion: These data suggest that at vStr activity reflects a mixture of cue identity and reward outcomes, encoded in partially overlapping populations, such that information about both can be extracted from the population as a whole. This arrangement may allow for flexibility in the decoding of multiple motivationally relevant signals in a context-dependent manner.

Disclosures: J. Gmaz: None. **J.E. Carmichael:** None. **M.A.A. van der Meer:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

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Program#/Poster#: 543.06/III13

Topic: H.01. Animal Cognition and Behavior

Title: Kappa opioid receptors modulate input interactions in the ventral striatum

Authors: *J. M. BROOKS[1], P. O'DONNELL[2];
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Abstract: Kappa opioid receptors (KOR) are highly enriched within the ventral striatum (VS) and are thought to modulate striatal neurotransmission. KOR activation inhibits local glutamatergic transmission via a presynaptic site of action, suggesting excitatory inputs to the VS are regulated by KOR. The VS receives glutamatergic input from multiple brain regions, including the prefrontal cortex (PFC) and hippocampus (HP), and individual medium spiny neurons (MSNs) serve as the site of convergence for these diverse inputs. Recent data indicates

competition can arise between these inputs. Previous data show robust PFC stimulation leads to a reduction in ongoing HP-evoked MSN responses, in part, through the recruitment of local inhibitory mechanisms in the VS. Here we explored the role of KOR activation in cortical suppression of competing excitatory synaptic inputs on VS MSNs. Whole-cell recordings were performed from rats receiving bilateral HP injections of a viral vector expressing channelrhodopsin 2 (ChR2) under the CamKinase II promoter. Input interactions were assed in MSNs through electrical stimulation of PFC fiber tracts and light stimulation of HP inputs expressing ChR2. Optogenetically-evoked HP EPSPs were greatly attenuated after a short latency (50 ms) following burst-like PFC stimulation (5 pulses, 20 Hz, 0.1-0.5 mA) and the magnitude of this suppression was partially reversed following bath application of picrotoxin (100 uM), but not saclofen (2 uM). As the reduction is not complete, we assessed the role KOR signaling. Although KORs are localized to the VS, their cellular distribution is not well understood. To determine whether KORs selectively modulate VS inputs, we examined the effect of KOR activation on HP and PFC- evoked EPSPs in MSNs. Superfusion of the KOR agonist U69593 decreased HP and PFC EPSP amplitudes, indicating KORs do not decrease excitatory neurotransmission in the VS in an input-selective manner. We next examined whether blockade of KOR signaling would impact PFC-induced suppression. Indeed, we found that bath application of the KOR antagonist norBNI (100 nM) decreased the magnitude of PFC-induced suppression of HP responses in a manner similar to that observed following GABA-A receptor blockade. Co-administration of norBNI and PTX blocked the suppressive effect of robust PFC stimulation on HP inputs to the VS. These findings confirm and extend our assertion that shifts in VS neuronal activity involve local suppression of competing afferents converging on the same MSN. Furthermore, these data indicate PFC-driven heterosynaptic suppression involves co-activation of local inhibitory mechanisms and opioid peptide signaling.

Disclosures: **J.M. Brooks:** A. Employment/Salary (full or part-time): Pfizer Inc. **P. O'Donnell:** A. Employment/Salary (full or part-time): Pfizer Inc..

Poster

543. Cognition: Corticostriatal Circuit and Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: H.01. Animal Cognition and Behavior

Support: CIHR (MOP-102662)

CFI

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Title: Neural dynamics of cortical and basal ganglia circuits during dynamic decision-making

Authors: *P. E. CISEK[1], D. THURA[2], J.-F. CABANA[1], A. FEGHALY[1];
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Abstract: How does the brain make decisions in a dynamic and constantly changing world? We have previously shown (Thura & Cisek, 2014, 2016) that when monkeys perform a task in which relevant sensory information for action selection varies over time and they are free to respond at any moment, neurons in dorsal premotor (PMd) and primary motor cortex (M1) continuously reflect the evolution of sensory information along with a context-dependent signal related to the growing urgency to respond. Approximately 280ms prior to movement onset, the activity of these cells reflects the resolution of a competition between the choices, implicating them in the process of volitional commitment. Here, we examine the interactions between PMd/M1 and two sources of input, from the dorsolateral prefrontal cortex (dlPFC) and from the external and internal globus pallidus (GPe/GPi). We analyze data by plotting the dynamical state of the system as a trajectory through a high-dimensional neural space defined by the activity of each recorded neuron. We then use Principal Components Analysis to reduce this high-D space into a lower-dimensional projection and examine how neural trajectories in different cortical and subcortical regions evolve over time in different conditions. We found that the strongest components identified by PCA are functionally interpretable, corresponding to (in order): the transition from deciding-to-acting, the sensory evidence, the urgency signal, and the monkey's speed-accuracy tradeoff policy. During deliberation, the neural state of PMd/M1 evolves on a 2-dimensional "decision manifold" defined by orthogonal directions roughly corresponding to sensory evidence and urgency, and then rapidly falls off this surface at the moment of commitment into trajectories specific to each target choice. In PFC, activity is primarily confined to a narrower manifold extended primarily along the evidence dimension. In sharp contrast to the cortical dynamics, activity in the GPe and GPi does not reflect deliberation and is instead confined to the urgency dimension, just until the putative moment of commitment, at which time it also falls into the choice-dependent attractors. These results suggest that the process of deliberation unfolds primarily in the cortical regions, driven by evidence from dlPFC and a non-specific urgency signal from GPi, until a critical point is reached in cortex, engaging tuning in the cortico-striatal loop and confirming the volitional commitment to the movement choice.

Disclosures: **P.E. Cisek:** None. **D. Thura:** None. **J. Cabana:** None. **A. Feghaly:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

Location: Halls B-H

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Program#/Poster#: 543.08/III15

Topic: H.01. Animal Cognition and Behavior

Title: Feature-specific reward prediction errors during reinforcement learning across nonhuman primate fronto-striatal circuits

Authors: *M. OEMISCH, S. WESTENDORFF, T. WOMELSDORF;
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Abstract: When only one stimulus feature among many predicts reward, it becomes pivotal for an agent to learn feature-specific reward predictions. This depends on updating existing predictions with signals reflecting the discrepancy of predicted and actually experienced reward outcomes. These prediction error (PE) signals are encoded in the firing of neurons across multiple areas of the fronto-striatal system, among others.
An unresolved question is where PEs are encoded that are not only indicating a general error, but are uniquely informative about the feature giving rise to the prediction error. Such feature specificity of PE signals could enhance learning efficiency in reinforcement learning models by allowing a more selective value-updating of synaptic weight.
To test the possibility of feature-specific signaling of PEs we recorded neuronal spiking activity across multiple areas of the macaque fronto-striatal system during a reversal learning task. In this task a monkey was presented with two moving grating stimuli that differed in color, location, and motion direction. The animal was rewarded for reporting the motion of the stimulus with the reward-associated color. The reward-associated color was reversed uncued between blocks. This setting allowed tracking the learning of feature-outcome associations independent from action- and location-outcome associations. To test for feature-specific and unspecific PE signals we extracted positive PEs as those outcome signals following rare correct outcomes during reversal learning. Negative PEs were identified as the rare erroneous outcomes after learning at stable performance. PE signals were identified as feature-specific when they were selectively larger for e.g. a specific rewarded color.
We found neurons encoding PE signals across all fronto-striatal areas. More neurons encoded positive than negative PEs, and the largest proportions of PE encoding neurons were in the striatum and lateral PFC. Importantly, a substantial proportion of PE signals was feature-specific; this proportion was larger in medial and lateral PFC compared to striatum and emerged earliest in lateral PFC compared to ventral striatum.
These findings suggest that prefrontal PE signaling encodes the specific features that led to an unexpected outcome, while striatal neurons encode a more general PE signal. These results illustrate that neurons encoding PEs carry dissociable content about the feature origin of this

error. We speculate that differently feature-tuned PE signals enhance the speed of learning by facilitating selective credit assignment during the updating of feature value predictions during goal-directed behavior.

Disclosures: **M. Oemisch:** None. **S. Westendorff:** None. **T. Womelsdorf:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

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Topic: H.01. Animal Cognition and Behavior

Support: F32MH110184

NIMH R01MH087542

Title: DREADD-mediated manipulation of the indirect pathway of the dorsomedial striatum disrupts flexible updating

Authors: ***K. DELEVICH**, L. WILBRECHT, L.-H. TAI, S. VEDULA, Y. ZHANG;
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Abstract: Successful goal-directed behavior requires the ability to flexibly select actions that result in reward and avoid actions that result in no reward, or worse, punishment. A lack of flexibility and insensitivity to negative outcomes is characteristic of multiple psychiatric disorders. The dorsal medial striatum (DMS) is thought play a critical role in supporting flexible behavior in response to negative and positive feedback. Previous data from our lab and others implicate the activity of D2 receptor-expressing medium spiny neurons (MSNs) of the DMS in action selection (Tai et al., 2015) avoidance behavior (Kravitz et al., 2012; Lee, Tai et al., 2015), and responses to negative feedback (Cox et al. 2015). To further investigate the role of D2 MSNs of the DMS in behavioral flexibility we employed the chemogenetic tool hM4Di to selectively inhibit D2 MSNs in the DMS in an odor based 4-choice reversal task (Johnson et al., 2011; 2016). Male D2-Cre mice were bilaterally injected into the DMS with 0.5 uL DIO-hM4Di or DIO-mCherry and after three weeks for recovery and viral expression were trained to find a reward in 1 out of 4 scented bowls. CNO (1 mg/kg) was then given the next day, just prior to a recall and reversal test where the contingency was changed to a new odor. The D2-Cre DIO-hM4Di group showed greater perseveration compared to DIO-mCherry controls, searching more frequently in the previously rewarded odor and taking more trials to begin exploring alternate

odor choices. These data suggest that activity in the DMS indirect pathway MSNs impacts the efficiency of flexible updating.

Disclosures: **K. Delevich:** None. **L. Wilbrecht:** None. **L. Tai:** None. **S. Vedula:** None. **Y. Zhang:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 543.10/III17

Topic: H.01. Animal Cognition and Behavior

Title: How do the subregions of striatum encode different value memories? - Brain-wide inputs to tail of caudate-putamen in rodent

Authors: *H. JIANG[1,2], H. Z. KIM[1,2], H. F. KIM[1,2];
[1]Ctr. for Neurosci. Imaging Res., Inst. For Basic Science(Ibs), Suwon-si, Korea, Republic of;
[2]Dept. of Biomed. Engin., Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Different subregions of striatum are involved in different value-coding and behaviors in primate and rodent systems (Kim & Hikosaka, 2013; Yin & Knowlton, 2006). Head and tail of caudate nucleus (CDh & CDt) is one of the good examples: fast neuronal plasticity and adaptive behavior by CDh vs. slow neuronal plasticity but long-lasting behavior such as habit by CDt. Then, which mechanism makes these differences? One hypothesis is that different brain inputs to CDh and CDt generate these plasticity differences. Indeed we previously found separate dopaminergic inputs to CDh and CDt (Kim et al., 2014), but their brain-wide inputs remain a question. Here we first identify the whole inputs to tail of caudate-putamen (tCPu) using rodent system.
To examine the brain-wide inputs, rhodamine-conjugated retrobead was injected into the tCPu of rat brain using stereotaxic coordinates. The tCPu Inputs from several brain systems were identified. First we found strong inputs from limbic system to tCPu; neurons in the entorhinal and perirhinal cortices strongly project to tCPu, and amygdala also has less but significant number of projecting neurons to tCPu. In the insular cortex neurons were labeled across wide regions of the structure, indicating their converging inputs to the tCPu. In visual system we found a few input from primary visual cortex; instead, neurons in the secondary visual cortex more strongly innervate tCPu. Similarly, in motor system we found more inputs from the secondary motor cortex to tCPu than the primary motor cortex. In the substantia nigra pars compacta (SNc), dopamine neurons in caudal-lateral region of SNc were mainly labeled, indicating the selective input of value information to tCPu as previously reported in primate and

rodent systems (Kim et al., 2014; Manegas et al., 2015).
Our data show limbic, insular, visual, motor and value inputs to tCPu in rodent system (please also see brain-wide inputs in macaque monkey by Griggs et al., 2016 SfN poster). Comparing the brain-wide inputs to head and tail of CPu would give a critical answer for understanding the mechanism of neuronal plasticity and behavioral differences in the CPu subregions.

Disclosures: H. Jiang: None. **H.Z. Kim:** None. **H.F. Kim:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

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Topic: H.01. Animal Cognition and Behavior

Support: The Intramural Research Program at the National Institutes of Health, National Eye Institute

Title: Direct and indirect pathways in basal ganglia signaling opposite reward values: anatomical study

Authors: *H. AMITA[1], H. F. KIM[3,4], W. GRIGGS[1], A. GHAZIZADEH[1], O. HIKOSAKA[1,2]; [1]Lab. Sensorimotor Research, NEI, NIH, Bethesda, MD; [2]Intramural Res. Program, NIDA, NIH, Baltimore, MD; [3]Dep. of Biomed. Engin., Sungkyunkwan Univ., Suwon, Korea, Republic of; [4]Ctr. for Neurosci. Imaging Res., Inst. for Basic Sci., Suwon, Korea, Republic of

Abstract: Striatal output neurons project to the output area of the basal ganglia (e.g., substantia nigra pars reticulata, SNr) directly (i.e. direct pathway), or indirectly (i.e. indirect pathway) through globus pallidus externus (GPe) and subthalamic nucleus (STN). This scheme has suggested that the direct and indirect pathways are separate and carry different signals. However, this hypothesis has not been tested systematically. In fact, some striatal neurons project to both SNr and GPe in rats, thus raising a question about the separate circuit scheme. No relevant data are available in primates.
To answer this question, we performed anatomical experiments in 2 steps. In Experiment 1, we chose one striatal area (caudate tail, CDt) and determined its exact targets within SNr and GPe. For this, we injected a tracer (CTB555) in CDt. We found anterograde labeled axon terminals that were localized in caudal-dorsal-lateral SNr (cdlSNr) and caudal-ventral GPe (cvGPe). In Experiment 2, we determined whether individual CDt neurons project to cdlSNr or cvGPe, or both. For this, we injected different tracers into cdlSNr (Fast Blue) and cvGPe (CTB555). We found retrograde labeled neurons that were localized in CDt and caudal-ventral putamen (cvPut)

from both cdlSNr and cvGPe. However, very few neurons in CDt and cvPut were double-labeled. These results indicate that direct and indirect pathways from CDt and cvPut are largely separate.
Experiment 2 also revealed an unexpected feature of the indirect pathway. It has been shown that STN is mutually connected with GPe and thus acts as a key station of the indirect pathway. Our results were different from this scheme. After CTB555 injection in cvGPe, we found neither anterograde labeled axon terminals nor retrograde labeled neurons in STN. Instead, anterograde labeled axon terminals were heavily localized in cdlSNr. These results suggest that the direct and indirect pathways of CDt send signals to the same output station (i.e. cdlSNr). The indirect pathway is detoured only through cvGPe, but not STN.
The anatomical separation of the direct and indirect pathways of CDt and cvPut would allow them to send completely different signals to the same basal ganglia output (cdlSNr). Indeed, our behavioral and electrophysiological experiments (poster by Kim, Amita and Hikosaka) found that both of these pathways carry reward value signals, but in opposite directions: Direct pathway - Positive, Indirect pathway - Negative.

Disclosures: H. Amita: None. **H.F. Kim:** None. **W. Griggs:** None. **A. Ghazizadeh:** None. **O. Hikosaka:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

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Program#/Poster#: 543.12/III19

Topic: H.01. Animal Cognition and Behavior

Title: Direct and indirect pathways in basal ganglia signaling opposite reward values: electrophysiological study

Authors: *H. F. KIM[1,2], H. AMITA[3], M. YASUDA[4], O. HIKOSAKA[3,5];
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Abstract: Neurons in the tail of caudate nucleus (CDt) in macaque monkeys encode stable reward values of visual objects and guide visual habit (Kim & Hikosaka, 2013). CDt neurons project to caudal-ventral region of globus pallidus externus (cvGPe) in indirect pathway and to caudal-dorsal-lateral region of substantia nigra pars reticulata (cdlSNr) in direct pathway (2016 SfN poster by Amita, Kim and Hikosaka). We hypothesized that different stable reward values

are separately processed in the indirect and direct pathway.
To test this hypothesis, we used electrophysiological methods. First, we electrically stimulated CDt while recording single neuronal activity in their target structures, GPe and SNr. 20 among 28 GPe neurons showed inhibitory responses, and the inhibited neurons were localized in cvGPe. CDt microstimulation also induced inhibitions in cdlSNr neurons (Yasuda & Hikosaka, 2015). These data indicate that CDt has inhibitory connections to both cvGPe and to cdlSNr. We then electrically stimulated cvGPe while recording neuronal activity in cdlSNr. cvGPe microstimulation induced inhibitions in cdlSNr neurons (n = 14), showing the inhibitory connection from cvGPe to cdlSNr. To further study the connection, we stimulated cdlSNr while recording single neuronal activity in cvGPe. We found that cvGPe neurons (n = 13) were activated antidromically. These results indicate that cvGPe neurons have monosynaptic connections to cdlSNr neurons.
Our data show two inhibitory pathways which would be involved in visual habit: direct pathway (CDtcvSNr) and indirect pathway (CDtcvGPecdlSNr). These two pathways were anatomically confirmed by our tracer study (2016 SfN poster by Amita, Kim and Hikosaka). Which value information is encoded in each pathway? We recorded activity of single cvGPe neurons while high- and low-valued fractal objects were presented. The fractal objects were learned over 4 days, and thus the monkeys developed a habitual preference of gazing without any reward outcome. Among 134 visual responsive neurons, 58 neurons differentially responded to previously learned objects. Notably, cvGPe neurons were mainly inhibited by low-valued objects. In contrast, cdlSNr neurons were mainly inhibited by high-valued objects (Yasuda & Hikosaka, 2015).
Our data indicate that opposite stable values are processed through indirect and direct pathways: (-) value in CDt Inhibit cvGPe Disinhibit cdlSNr vs. (+) value in CDt Inhibit cdlSNr. Consequently, neurons in superior colliculus (SC) are inhibited by low-valued object and disinhibited by high-valued objects, causing habitual saccades to high-valued objects.

Disclosures: **H.F. Kim:** None. **H. Amita:** None. **M. Yasuda:** None. **O. Hikosaka:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

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Topic: H.01. Animal Cognition and Behavior

Support: The Intramural Research program at the National Institutes of Health, National Eye Institute

Title: Different inputs to caudate head and tail for different types of reward values

Authors: W. GRIGGS[1], H. F. KIM[2,3], A. GHAZIZADEH[1], *O. HIKOSAKA[1,4];
[1]Lab. Sensorimotor Res., Natl. Eye Inst., Bethesda, MD; [2]Dep. of Biomed. Engin., Sungkyunkwan Univ., Suwon, Korea, Republic of; [3]Ctr. for Neurosci. Imaging Res., IBS, Suwon, Korea, Republic of; [4]Intramural Res. Program, Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Anatomically distinct pathways originating from the caudate head (CDh) and caudate tail (CDt) encode flexible- and stable-valued memories respectively (Hikosaka et al., Annu Rev Neurosci 2014), but the inputs to these value-coding areas are not well understood. To identify these inputs, we first located flexible and stable value-coding areas in CDh and CDt of two rhesus macaque monkeys by showing them fractal objects with different amounts of reward reversibly or consistently. We then injected different retrograde tracers into these areas: Diamidino Yellow into CDh and CTB555 into caudal CDt of both monkeys, and CTB488 and Fast Blue into rostral CDt of monkey Z and S respectively. We found that CDh and CDt receive differential inputs from several subcortical and cortical areas including amygdala, claustrum, thalamic nuclei, presubiculum, insula, superior temporal sulcus (STS), and area TE. Within the amygdala, there are strong projections from the basal lateral nucleus to CDt, but no projections to CDh. The claustrum also projects to CDt, but not to CDh. Within the thalamus, the paraventricular and medial dorsal nuclei project to CDh while the pulvinar nucleus projects to CDt. There are projections from the parafascicular nucleus to both CDh and CDt, but the projecting areas are spatially distinct. Near the hippocampus, there are strong projections from the pre- and para-subiculum to CDt, but no projections to CDh. Within the cortical regions, there are projections from the insula to CDh, but not to CDt. Within the STS, there are projections to CDh and CDt, but they originate from different cortical layers and spatially distinct subregions. We also found strong projections from areas TE and TEO, including scene-coding areas, to CDt, with weak projections to CDh. So far, we found no neuron projecting to both CDh and CDt. Our data indicate that CDh and CDt constitute anatomically separate circuits, both for their outputs and inputs. Their outputs (aiming at the superior colliculus through the substantia nigra pars reticulata) encode strong, but distinct, value memories. It is likely that many of their inputs (shown in this study) also encode value memories (see companion abstract, Ghazizadeh et al.). Understanding the role of each of these input areas is important for understanding how value memories are created and stored.

Disclosures: W. Griggs: None. **H.F. Kim:** None. **A. Ghazizadeh:** None. **O. Hikosaka:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

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Topic: H.01. Animal Cognition and Behavior

Support: F32 MH107206

Title: Pathway-specific coding of action and value in the dorsomedial striatum

Authors: *C. H. DONAHUE[1], M. LIU[2], A. C. KREITZER[2];
[1]Neurosci., [2]The Gladstone Inst., San Francisco, CA

Abstract: The basal ganglia play a critical role in action selection and reinforcement learning, and have been shown to represent action values which may help guide decision making. The striatum contains two intermingled classes of projection neurons, the direct and indirect pathway medium spiny neurons (dMSNs and iMSNs), that exert opposing effects on downstream structures. While the role of the direct and indirect pathways in facilitating and suppressing action are well known, the pathway-specific contributions to reinforcement learning are much less understood. To address this question, we trained mice to perform a value-based dynamic foraging task in a three-port chamber. The animals were trained to initiate a trial in the center port and to make a choice with a nose poke to the left or right port. The ports were baited with different probabilities of reward delivery (either 60% or 15%) to elicit matching behavior. The location of the port associated with high reward probability underwent frequent un-signaled reversals so that the animals had to learn by integrating their recent history of actions and outcomes.

We imaged pathway-specific activity in the dorsomedial striatum through the use of a genetically encoded calcium indicator (GCaMP6m) with a head-mounted microscope while the animals performed the task. As has been shown previously, analysis of bulk fluorescence showed that both dMSNs and iMSNs selectively responded when the animals made movements that were contralateral to the recording site. Contralaterally-directed movements from the side port towards the center port (during the trial reinitiation period) were associated with significantly stronger activity than movements from the center towards the side ports (during the decision period). Single-cell responses during trial reinitiation revealed this was due to distinct subpopulations of dMSNs and iMSNs that selectively combined information about the most recent action and outcome. A significantly larger population of dMSNs were active following evidence that the contralateral port was of higher value, while iMSNs were more active following evidence that the ipsilateral port was of higher value. These results provide evidence that the direct and indirect pathways play opposing roles in value-based learning.

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Poster

543. Cognition: Corticostriatal Circuit and Physiology

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Topic: G.01. Appetitive and Aversive Learning

Support: Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Different population of primate caudate neurons is involved in decision making under different emotional context.

Authors: *Y. UEDA, K. NAKAMURA;
Kansai Med. Univ., Osaka, Japan

Abstract: Neuronal mechanisms of decision making have been investigated for reward-oriented behavior, mostly choice of reward vs. no reward, and the basal ganglia (BG) have been implicated in the process. However, choice of a reward against a punishment or no-reward against punishment (avoidance) also occurs. We hypothesized that such inclusion of an aversive option creates specific emotional context, referred as stress or anxiety, and that distinct neuronal circuit may be comprised. To address this issue, we designed a behavioral paradigm in which animals made choice under different emotional context. Two monkeys (*Macaca fascicularis* and *Macaca mulatta*) were conditioned for three fractal images, with a juice (R), a neutral tone (T), or an aversive airpuff (A). After fixating on a central fixation point (FP, 1000 ms), a pair of images (R-T, R-A, T-A) appeared in the left and right of the FP. The monkeys chose one of the images by eye movements to obtain a reward and/or to avoid a punishment. The same image pair was repeated up to 30 trials as a block while the side of the images changed randomly, which allowed the animals to predict the specific pair before its presentation. Block-based comparison revealed significantly more frequent suboptimal choices for pairs with ‘A’ (R-A, T-A) than that without ‘A’ (R-T). Low nasal temperature for the pairs with ‘A’ indicated sympathetic dominance. Trial-based analyses also showed sympathetic dominance; higher heart rate before target presentation, for following suboptimum choices. Thus, inclusion of ‘A’ induced emotional changes, assessed objectively by the autonomic responses, and impaired the decision process. To investigate the neuronal substrate of decision making when an aversive outcome was possible, we measured neuronal activity of the caudate, an input channel of the BG for this task in the same animals. During the FP period where emotional context was known during the preparation for the choice, 198 out of 374 (53%) neurons exhibited differential activity depending on the pair type. Among them, 70 showed stronger activity for the pairs with 'R' (R-T, R-A) than those without 'R' (T-A), consistent with the hypothesis that the striatum is involved in reward-dependent modulation in action. However, 85 showed stronger activity for the pairs with 'A' than

those without 'A' suggesting aversive-context encoding. The aversive-context preferred activity did not simply encode ‘punishment’ because their activity was higher for trials with optimum choices than those with suboptimum choices. These results suggest that different subsets of primate caudate neurons are recruited in decision making under different emotional context.

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Poster

543. Cognition: Corticostriatal Circuit and Physiology

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC

Dartmouth College

CIHR Post_Doctoral Fellowship

Title: Unilateral naris occlusion suppresses gamma oscillations in ventral but not dorsal limbic structures in the rat

Authors: *J. E. CARMICHAEL[1], J. Y. KHOKHAR[2], A. I. GREEN[2], M. A. A. VAN DER MEER[1];
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Abstract: Oscillations in the local field potential (LFP) are thought to reflect aspects of information processing, such as the gating or binding information from anatomically distant regions. In the rodent limbic system, interconnected regions such as the prelimbic frontal cortex (PL), orbitofrontal cortex (OFC), ventral striatum (vStr), and cingulate cortex (CG), show coherent oscillations and spike-field relationships in various frequency bands, which may mediate the flow of information between these areas.
Most of these areas are anatomically proximal to the piriform cortex (PC) and olfactory bulb (OB), which are located on the ventrolateral side of the rat brain, and show strong gamma-band oscillations. Our previous work has shown that inactivation of the OB/PC by unilateral naris occlusion abolishes gamma oscillations in the ipsilateral vStr LFP. This result suggests that gamma oscillations seen in the vStr LFP are actually volume-conducted from OB/PC, a conclusion further supported by clear gradients in gamma power that increase towards the ventrolateral vStr (Carmichael et al. 2015). Other structures adjacent to the OB/PC, such as the

OFC, also display gamma oscillations, suggesting that the gamma oscillations seen in the OFC field potential may contain contributions from the OB/PC, and raises the question of how far into adjacent structures OB/PC oscillations continue to shape the field potential.
We recorded local field potentials from rats (n = 4) implanted with electrodes in OFC, vStr, PL, and CG, as they underwent reversible unilateral naris occlusions. Both the vStr and OFC showed strong suppression in gamma power only when the ipsilateral naris was blocked, suggesting that local gamma oscillations in these structures are likely volume-conducted from the adjacent OB/PC. In contrast, the PL and CG instead showed similar gamma power during pre/post occlusion as well as contra-/ipsilateral occlusions.
Thus, gamma oscillations seen in brain regions adjacent to the OB/PC are likely not generated locally; however, the influence of OB/PC gamma does not extend to more distal, yet anatomically connected, areas such as PL and CG. This result does not imply that these volume-conducted components of the field potential are unimportant or uninformative; neurons in the vStr and OFC show striking phase-locking to gamma-band oscillations in the LFP. Rather, this emerging view of gamma oscillations in ventro-lateral limbic circuits highlights the importance of the PC common input as a major influence, perhaps excluding the possibility that areas such as vStr and OFC can control oscillations in their LFP.

Disclosures: **J.E. Carmichael:** None. **J.Y. Khokhar:** None. **A.I. Green:** None. **M.A.A. van der Meer:** None.

Poster

543. Cognition: Corticostriatal Circuit and Physiology

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 543.17/III24

Topic: H.01. Animal Cognition and Behavior

Support: G. Harold and Leila Y. Mathers Charitable Foundation

William and Jane Walsh Charitable Remainder Unitrust

Title: Biologically plausible learning in recurrent neural networks

Authors: *T. MICONI;
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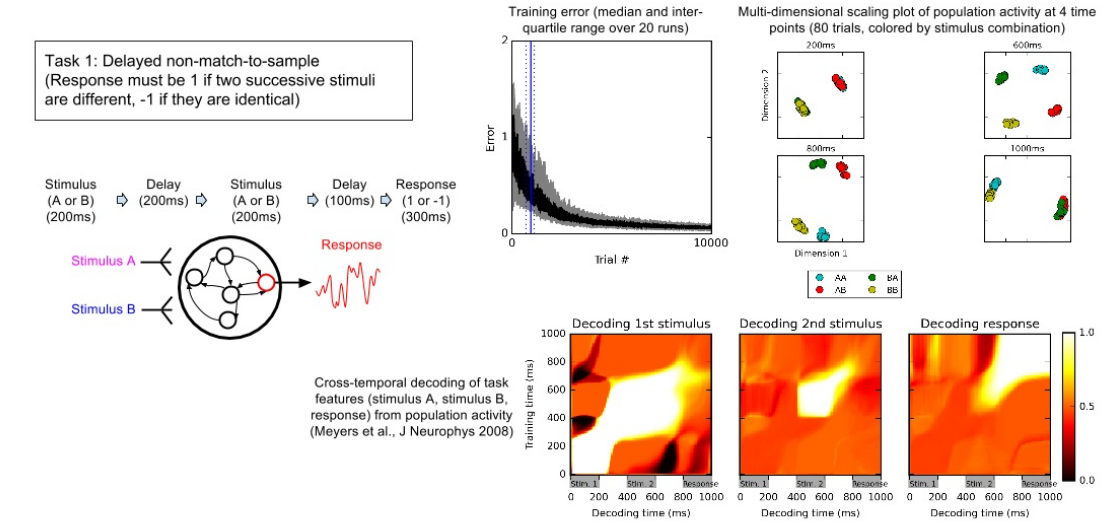
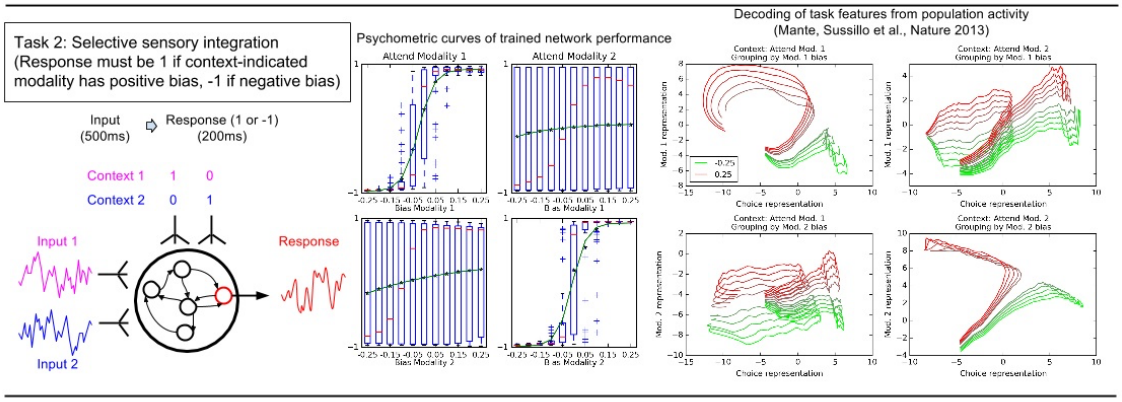
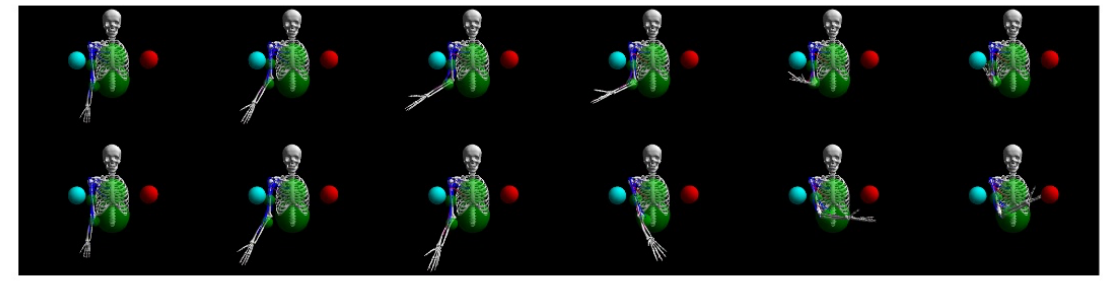
Abstract: Recurrent neural networks exhibit complex dynamics, reminiscent of cortical activity in higher areas during behavioral tasks. However, existing methods for training such networks are either biologically implausible, or require a real-time continuous error signal to guide the

learning process. This is in contrast with most behavioral tasks, which only provide time-sparse, delayed rewards. Here we introduce a biologically plausible learning algorithm, that can train chaotic recurrent networks based solely on delayed, phasic reward signals at the end of each trial. The method requires no dedicated feedback or readout networks: the entire network connectivity is subject to learning based on exploratory perturbations, and the network's output is read from one arbitrarily chosen network cell. We apply this method to learn three different tasks: a delayed nonmatch-to-sample task (requiring memory maintenance and non-linear selectivities); a selective attention task, in which the network learns to flexibly attend to one of two concurrent input streams while ignoring the other; and a motor control task using a bio-mechanical model of the human arm, with 4 degrees of freedom actuated by 16 independent muscles, to produce reaching movements toward instructed targets, showing that the algorithm can learn to coordinate multiple outputs. The trained networks exhibit complex dynamics previously observed in animal cortex, such as dynamic encoding and maintenance of task features, switching from stimulus-specific to response-specific representations, and selective integration of relevant input streams. We conclude that recurrent neural networks, trained with reward-modulated Hebbian learning, offer a plausible model of cortical dynamics during both learning and performance of flexible behavior.







Disclosures: T. Miconi: None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 544.01/III25

Topic: G.02. Motivation

Support: CIHR

Title: Investigating the ascending and descending prefrontal-midbrain projections using optogenetics: Implications for the regulation of motivation for sustained effort.

Authors: *A. HARATIKIA[1], B. CARACHEO[1], N. GORELOVA[1], K. DEISSEROTH[2], J. K. SEAMANS[1];
[1]Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada; [2]Dept. of Bioengineering, Dept. of Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA

Abstract: Dopamine (DA) neurons of the ventral tegmental area (VTA) exert critical modulatory effects on target neurons in the prefrontal cortex (PFC). In a reciprocal manner, DA neurons are themselves targeted by glutamate projections from PFC neurons. While the function of this reciprocal pathway is not known, we hypothesized that events signaling effort may be encoded by the PFC neurons that project to the VTA and that the activation of VTA neurons may in turn release DA in the PFC which maintains attention during long bouts of sustained effort. The present study investigated the effects of optogenetic manipulations of VTA-PFC or PFC-VTA pathways during a modified progressive ratio (PR) task. In the first experiment, TH::Cre rats received VTA injections of a viral vector containing the Cre-inducible ChR2 gene. They were then implanted with optic fibers in the PFC and trained on a PR task where the required number of lever presses increased by 2 on each iteration and the rats were given as many chances as they desired to complete a ratio, so long as they responded at least once within a 2min. interval. If they did not respond within the 2min., this was taken as the breakpoint. We found that tonic 3Hz optic pulses within the PFC extended the breakpoint and made rats more willing to make subsequent attempts after a failure. These effects were reproduced by injections of amphetamine into the PFC. In contrast, phasic stimulation (5 x 20Hz) of the PFC either at the time of the lever presses or at the completion of a ratio, had no effect. A second series of experiments focused on the PFC-VTA pathway. Normal rats received PFC injections of a viral vector carrying ChR2 gene to produce pyramidal cell type specific expression under the control of the CAMKII promotor. These rats were then implanted with a tetrode array in the PFC for multiple single unit recordings as well as optic fibers in the VTA. In this case, VTA light pulses were not used to modify behavior but as a means to antidromically identify PFC-VTA projecting neurons. A subgroup of PFC neurons identified as projecting neurons responded in a relatively continuous manner during lever press bouts and stopped firing when a ratio was completed and

the lever retracted. Finally, a separate group of rats received PFC injections of a viral vector carrying the inhibitory opsin eNpHr3.0. In these animals, tonic light pulses in the VTA reduced break points. The results are therefore consistent with the hypothesis that subpopulations of PFC-VTA projection neurons are activated by prolonged efforts and these neurons may activate the VTA-PFC pathway and release DA in the forebrain which in turn serves to increase the propensity to sustain effort, especially after failure.

Disclosures: A. Haratikia: None. **B. Caracheo:** None. **N. Gorelova:** None. **K. Deisseroth:** None. **J.K. Seamans:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 544.02/III26

Topic: G.02. Motivation

Support: NIH NIDA F31 DA041104

Title: Coordination of brain wide activity dynamics by dopaminergic neurons

Authors: *H. K. DECOT[1], V. M. K. NAMBOODIRI[1], W. GAO[1], J. A. MCHENRY[1], J. H. JENNINGS[1], S.-H. LEE[1], P. A. KANTAK[1], Y.-C. KAO[1], M. DAS[1], I. B. WITTEN[2], K. DEISSEROTH[3], Y.-Y. SHIH[1], G. D. STUBER[1];
[1]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Princeton Univ., Princeton, NJ; [3]Stanford Univ., Stanford, CA

Abstract: Addiction may arise in part from dysregulated activity of ventral tegmental area dopaminergic (TH^{VTA}) neurons, as well as from more global maladaptation in neurocircuit function. The direct consequences of dopamine signaling are largely restricted to brain regions that contain appreciable presynaptic fibers that release dopamine, as well as postsynaptic dopamine receptors. However, dopaminergic signaling may also indirectly influence the activity in multiple brain regions, some of which may receive little to no direct dopamine input. Here, we studied how *in vivo* modulation of TH^{VTA} neurons altered brain-wide activity patterns using optogenetics coupled with fMRI. To target DA neurons within the midbrain, tyrosine hydroxylase (TH)-Cre adult Long Evans rats were microinjected into the ventral tegmental area (VTA) with a Cre-inducible adeno-associated virus carrying the gene encoding channelrhodopsin-2 (ChR2), a light-gated cation channel fused to an enhanced yellow fluorescent protein (EYFP) (TH^{VTA}::ChR2 rats) or only EYFP (TH^{VTA}::control rats). Chronic optical fibers were stereotactically implanted bilaterally above the VTA to selectively activate

DA neurons within this region. fMRI experiments were performed 5-6 weeks after surgery. Single shot, single sampled GE-EPI sequences (BW= 300 kHz, TR= 1000 ms, TE= 8.107 ms, 80x80 matrix, FOV= 2.56 x 2.56 cm2, slice thickness= 1 mm) were acquired using a Bruker 9.4T MR scanner and home-made surface coil. Applying a standard generalized linear model (GLM) analysis approach, our results indicate that selective optogenetic stimulation of TH^{VTA} neurons enhanced cerebral blood volume (CBV) signals in striatal target regions in a dopamine receptor dependent fashion. However, brain-wide voxel-based principal component analysis (PCA) of the same dataset revealed that dopaminergic modulation activates several additional anatomically distinct regions throughout the brain, not typically associated with dopamine release events. Furthermore, explicit pairing of TH^{VTA} neuronal activation with a forepaw stimulus of a particular frequency expanded the sensory representation of that stimulus, not exclusively within the somatosensory cortices, but brain-wide. These data suggest that VTA dopaminergic neuronal activity has immediate global consequences, and can remodel brain-wide neuronal activity patterns alone or in the presence of a sensory stimulus.

Disclosures: **H.K. Decot:** None. **V.M.K. Namboodiri:** None. **W. Gao:** None. **J.A. McHenry:** None. **J.H. Jennings:** None. **S. Lee:** None. **P.A. Kantak:** None. **Y. Kao:** None. **M. Das:** None. **I.B. Witten:** None. **K. Deisseroth:** None. **Y. Shih:** None. **G.D. Stuber:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 544.03/III27

Topic: G.02. Motivation

Support: John P. Stock Fellowship

Brain & Behavior Research Foundation

Brain Research Foundation

Title: Input-specific mechanisms of drug-evoked plasticity in the mesolimbic dopamine system

Authors: *J. W. DE JONG, S. A. AFJEI, J. R. PECK, I. CERNIAUSKAS, V. HAN, S. LAMMEL;
Mol. and Cell Biol., UC Berkeley, Berkeley, CA

Abstract: Drugs of abuse can hijack synaptic plasticity mechanisms in brain circuits that are central to reward processing. One of the most well established synaptic modifications caused by *in vivo* administration of drugs of abuse is an increase in the strength of excitatory synapses onto

ventral tegmental area (VTA) dopamine (DA) neurons. We have previously shown that a single injection of cocaine induces a strong and long-lasting (>21 days) potentiation of excitatory synapses onto Ih-lacking VTA DA neurons projecting to the medial shell of the nucleus accumbens (DA→mShell). However, the anatomical origin of the inputs to this population of neurons which undergo synaptic potentiation following cocaine administration is largely unknown. Two prominent inputs to the VTA, which have been associated with reward-related behaviors, arise from the lateral hypothalamus (LH) and dorsal raphe (DR). Thus, we hypothesized that *in vivo* cocaine exposure will induce synaptic adaptations in one or both of these pathways. By combining optogenetics with synaptic electrophysiology, we found that light stimulation of LH and DR terminals in the VTA induced robust excitatory postsynaptic currents (EPSCs) in DA→mShell neurons. We next assessed the synaptic strength of each pathway by measuring the relative contributions of AMPA receptors (AMPARs) and NMDA receptors (NMDARs). We found that a single *in vivo* injection of cocaine (15 mg/kg, IP) increased the synaptic strength of DR inputs in a small proportion of DA→mShell neurons when assessed 24 hours later. However, a robust increase in the AMPAR/NMDAR ratio of DR inputs to DA→mShell neurons was observed following 5 consecutive days of cocaine treatment. In addition, we found that the rectification index (RI) of the AMPAR current was significantly increased, suggesting an increase in GluR2-lacking AMPARs, which show a characteristic inwardly rectifying current at positive potentials. Surprisingly, however, when stimulating LH inputs to DA→mShell neurons, a single injection of cocaine induced a significant decrease in the AMPAR/NMDAR ratio and no change in the RI. Collectively, these results suggest that opposing forms of drug-evoked plasticity in distinct VTA afferent pathways may contribute to the reorganization of neural circuitry during early stages of drug addiction.

Disclosures: **J.W. De Jong:** None. **S.A. Afjei:** None. **J.R. Peck:** None. **I. Cerniauskas:** None. **V. Han:** None. **S. Lammel:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 544.04/III28

Topic: G.02. Motivation

Support: Whitehall Foundation

John P. Stock Fellowship

Regents' Junior Faculty Fellowship

Title: Dissecting lateral habenula circuits underlying depression-related behaviors

Authors: I. CERNIAUSKAS, J. W. DE JONG, N. SUNKARA, J. R. PECK, *S. LAMMEL; Mol. and Cell Biol., UC Berkeley, Berkeley, CA

Abstract: Recent research has indicated that the lateral habenula (LHb) may be a key region in the pathophysiology of depression. It has been shown that LHb neurons are hyperactive in depression-like states, and LHb lesions in rodents have led to a reduction of depression-related behaviors. However, little is known about the circuit and synaptic mechanisms that contribute to the hyperactivity of LHb neurons in depression-like states. Using a combination of anatomical tracing techniques, optogenetics and electrophysiology, we investigated afferent inputs to a specific subpopulation of LHb neurons which project to the ventral tegmental area (LHb→VTA). We found that chronic mild stress (CMS) induced depression-like behaviors in adult mice accompanied by significant increases in the firing rate of LHb→VTA neurons as revealed by whole-cell patch clamp recordings in acute brain slices. Next we employed a transsynaptic rabies virus strategy to further dissect the global input connectivity of this subpopulation. We found that major excitatory inputs to LHb→VTA neurons arise from the lateral hypothalamus (LH), entopeduncular nucleus (EP) and the VTA itself. These findings raise the possibility that synaptic changes in VTA, EP and/or LH excitatory inputs may drive hyperactivity of LHb→VTA neurons in depression-like states. To further characterize the synaptic properties of these inputs, we expressed channelrhodopsin-2 in the VTA, EP or LH and injected fluorescent RetroBeads into the VTA. We then recorded light-evoked excitatory postsynaptic currents by illuminating terminals from identified inputs to LHb→VTA neurons. Our results demonstrate that excitatory transmission of VTA and EP inputs to LHb→VTA neurons mainly relies on the presence of AMPA receptors and a relatively small NMDA receptor component. In contrast, LH inputs exhibit a more pronounced synaptic NMDA receptor component. We are currently investigating whether CMS leads to input-specific synaptic alterations of VTA, EP and LH afferents to LHb→VTA neurons. The identification of specific inputs that drive hyperactivity of LHb→VTA neurons and the underlying synaptic mechanisms will reveal important insights into the pathophysiology of depression, which may lead to novel circuit-specific therapeutic interventions.

Disclosures: I. Cerniauskas: None. **J.W. de Jong:** None. **N. Sunkara:** None. **J.R. Peck:** None. **S. Lammel:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

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Program#/Poster#: 544.05/III29

Topic: G.02. Motivation

Support: HFSP CDA00029/2013

FWO 11ZA317N

Marie-Curie 618683

Title: Responses of VTA/SNc neurons to stimulus novelty

Authors: J. MORRENS, J. NOUTEL, *S. HAESLER;
Neuroelectronics Res. Flanders, Leuven, Belgium

Abstract: The ability to discriminate novel from familiar sensory stimuli is a fundamental feature of the central nervous system. When humans or animals detect novel stimuli in their environment, they respond with distinct orienting and exploratory behaviors. Novel stimuli familiarize after a few exposures suggesting a very rapid form of memory formation. Disturbances in novelty processing are associated with numerous human pathological conditions including schizophrenia and autism, which highlights the clinical relevance of understanding the circuit mechanisms underlying novelty detection and familiarization. A candidate circuit implicated in novelty processing resides in the two dopaminergic midbrain structures substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). Compared to the evidence linking dopamine with reinforcement learning, knowledge about the role of dopamine neurons in novelty processing is sparse. Human and animal suggest activation of midbrain dopamine neurons by stimulus novelty, but the response properties of dopamine single-units have yet to be systematically investigated under experimental conditions, in which stimulus novelty is manipulated selectively. Here, we used a recently developed novel behavioral paradigm to investigate the firing properties of optogenetically identified DA neurons to stimulus novelty. To relate novelty responses of dopamine neurons to their well-described responses to reward and punishment, we also recorded them in a reward/punishment task in the same behavioral session after animals have completed the novelty task. We compared response dynamics between neural and behavioral responses. Moreover, we compared responses between dopamine neurons in the SNc and VTA. Given that both structures differ considerably in their projection targets and electrophysiological properties, our results have implications for where novelty signals are broadcasted. In our ongoing work, we use optogenetic techniques to investigate the causal contribution of dopamine neurons to novelty processing.

Disclosures: **J. Morrens:** None. **J. Noutel:** None. **S. Haesler:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

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Program#/Poster#: 544.06/III30

Topic: G.02. Motivation

Support: NIH Grant NS23805

Title: Projection to the rostromedial tegmental nucleus from the lateral preoptic area and ventral pallidum probed with Fos immunoreactivity following administration of amphetamine

Authors: *D. S. ZAHM, Y. TAN, K. P. PARSLEY;
Pharmacol. and Physiological Sci., St. Louis Univ. Sch. of Med., Saint Louis, MO

Abstract: The rostromedial tegmental nucleus (RMTg), a part of the medial mesopontine reticular formation comprising exclusively GABAergic neurons that project densely onto midbrain dopamine neurons, is widely acknowledged as a negative modulator of mesotelencephalic dopamine neuron activity in response to reward omission and aversion (Neuron 61:786-800, 2009; JCN 519:1143-1164, 2011; J Neurosci 31:11457-11471, 2011). Alternatively, infusion of the $GABA_A$ receptor agonist muscimol into the RMTg produces robust locomotor activation (Neuropsychopharmacol 40:676-87, 2015) and lesions of the RMTg facilitate motor learning (Neuropsychopharmacol 39:2788-98, 2014). Injection of anterograde tracer into the transition between the lateral preoptic area and caudomedial ventral pallidum (LPO-VP) produces dense anterograde labeling in the RMTg (Soc Neurosci Abstr 79.09, 2011) and infusion of $GABA_A$ antagonists, such as picrotoxin or bicuculline, centered on LPO-VP produce robust locomotor activation (Brain Struct Funct 219:511-526, 2014). These observations support a hypothesis that stimulation of LPO-VP inhibits the RMTg, which relieves midbrain dopaminergic neurons from RMTg-mediated inhibition. Consistent with this idea, we speculate that LPO-VP may also be activated (and RMTg in turn inhibited) in conditions of increased extracellular concentration of dopamine in the accumbens by inhibition of LPO-projecting ventral striatal GABAergic dopamine D2 receptor-expressing 'indirect pathway'-type medium spiny neurons, which are reported to be inhibited by dopamine (e.g., JPET 251:833-9, 1991; J Neurosci 14:7735-46, 1994). To address this possibility, we injected the retrograde tracer cholera toxin β subunit (Ctβ) into the RMTg of rats and after a week injected them with D-amphetamine (1 mg/kg) 2 hours prior to anesthetizing them, perfusing them with buffered aldehydes, sectioning the brains and processing the sections with antibodies against Ctβ and the immediate-early gene product, Fos. Retrogradely labeled neurons with and without Fos-expression were mapped with Neurolucida in sections containing LPO-VP. We consistently observed a small population (< 1% of retrogradely labeled) of small (10-15 μm) double-labeled neurons. Large, 'principal' projecting neurons were almost never double-labeled. Whereas, the results of the

experiment do not obviously support our hypothesis, we do identify a small population of 'activated' RMTg-projecting small neurons in LPO-VP. Small numbers of small neurons, e.g., the RMTg itself, can exert profound inhibition. Whether these, or other neurons not expressing Fos, are GABAergic and do remains to be determined.

Disclosures: D.S. Zahm: None. **Y. Tan:** None. **K.P. Parsley:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

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Program#/Poster#: 544.07/III31

Topic: G.02. Motivation

Support: DA037327

Title: Neural circuitry driving rostromedial tegmental nucleus (RMTg) responses to aversive stimuli

Authors: *H. LI, M. EID, N. PULLMANN, J. THOMPSON, T. C. JHOU;
Med. Univ. of South Carolina, Charleston, SC

Abstract: The rostromedial tegmental nucleus (RMTg) is a GABAergic nucleus that sends strong inhibitory projections to DA neurons, and acts as a "braking" system for DA neurons. RMTg neurons are activated by aversive stimuli and by cues that predict aversive outcomes, while inactivation or lesions of the RMTg greatly reduce many behavioral responses to these stimuli. The RMTg is known to receive a wide range of afferents, but it is not known how these afferents modulate RMTg responses to aversive stimuli or cues. Furthermore, it is not known whether different types of aversive stimuli or cues activate the RMTg by different pathways. Using *in vivo* electrophysiology we found that RMTg neurons were activated by a variety of aversive stimuli including footshock, loud tone, bright light and LiCl injection. By using rabies tracing, we identified a di-synaptic pathway from the entopeduncular nucleus (EPN) to the RMTg via the lateral habenula (LHb). We found that footshock and/or cocaine injections induced cFos expression in LHb-projecting EPN neurons, while unilateral EPN lesions blocked the stimulus-induced cFos increases in the ipsilateral LHb and RMTg. *In vivo* electrophysiology recording indicated that a large portion of EPN and LHb neurons exhibited activation by shock predictive cues and inhibition by cues predicting rewarding sugar pellets. Temporal inactivation of the EPN or LHb with GABA agonists reduced activation to shocks in RMTg neurons. Moreover, both RMTg and EPN neurons showed bi-phasic responses to cocaine, i.e. a reduced firing rate during roughly the first 10 mins and augmented firing rate during the subsequent 10

mins. This result is consistent with the immediate rewarding and delayed aversive effects of cocaine. Additionally, bilateral EPN inactivation blocked the acquisition of avoidance responses to cocaine on a runway test.

Disclosures: **H. Li:** None. **M. Eid:** None. **N. Pullmann:** None. **J. Thompson:** None. **T.C. Jhou:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 544.08/III32

Topic: G.02. Motivation

Support: NIDA Grant R01 DA038599

Title: Effects of chemogenetic manipulations of prelimbic inputs to the paraventricular nucleus of the thalamus on dopamine release in the nucleus accumbens of sign-trackers and goal-trackers.

Authors: *P. CAMPUS[1], Y. KIM[2], A. PARSEGIAN[1], I. R. COVELO[1], S. M. FERGUSON[3], M. SARTER[2], S. B. FLAGEL[1];
[1]Psychiatry, Univ. of Michigan Dept. of Psychiatry, Ann Arbor, MI; [2]Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI; [3]Dept. of Psychiatry and Behavioral Sci., Univ. of Washington, Seattle, WA

Abstract: Conditioned stimuli (CS) associated with rewards can acquire not only predictive value, but also incentive motivational value. When a cue is attributed with incentive motivational value or incentive salience, it gains the ability to control behavior, leading in some cases to maladaptive outcomes. However, individuals vary considerably in the extent to which they attribute motivational value to CS. In a Pavlovian Conditioned Approach (PCA) task, in which the presentation of a lever is immediately followed by the delivery of a food reward, some rats preferentially approach the lever (sign-trackers, STs) while others approach the food cup (goal-trackers, GTs). Importantly, while the lever is a predictor for both STs and GTs, only for STs does it become an "incentive stimulus". It has been previously shown that the sign-tracking response is dopamine (DA) dependent, but the goal-tracking response is not. Further, when the discrete cue previously paired with food is presented, it engages different neural circuitry in sign-trackers vs. goal-trackers, suggesting that while STs rely primarily on subcortical mechanisms, GTs utilize more cortical engagement. Recent work from our lab suggests that the paraventricular nucleus of the thalamus (PVT) may represent a central node that integrates

subcortical and cortical input differentially in STs and GTs. Together, these data led us to hypothesize that in GTs, input to the PVT from the prelimbic cortex (PrL) may serve to suppress subcortical processes critical for the expression of sign-tracking behavior. In support, preliminary data indicates that the activation of this PrL-PVT circuit using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) decreases sign-tracking behavior while the inhibition of the same pathway has the opposite effect. Here, we investigated the effects of selective manipulation of the PrL-PVT pathway on extracellular levels of DA in the NAc of STs and GTs. To this end, we used a dual viral vector approach to selectively express stimulatory Gq- or inhibitory Gi/o- DREADDs in PrL to PVT projecting neurons. Clozapine-N-oxide (CNO) was administered to activate DREADDs during PCA and levels of DA and other neurotransmitters in the nucleus accumbens were subsequently assessed using in vivo microdialysis. We found that, in STs, turning on" the PrL-PVT pathway appears to enhance DA in the NAc while, in GTs, "turning off" the PrL-PVT pathway appears to attenuate acetylcholine levels, without affecting DA. These studies may help to better understand the dynamics of the interaction between bottom-up and top-down processes involved in the neural circuitry that regulates cue-motivated behaviors.

Disclosures: P. Campus: None. **Y. Kim:** None. **A. Parsegian:** None. **I.R. Covelo:** None. **S.M. Ferguson:** None. **M. Sarter:** None. **S.B. Flagel:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

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Program#/Poster#: 544.09/III33

Topic: G.02. Motivation

Support: NIDA-IRP

NINDS-IRP

European Research Council ERC-2011-ADG-294313

Title: Glutamatergic neurons are intermixed with midbrain dopaminergic neurons in nonhuman primates and humans

Authors: *D. H. ROOT[1], H.-L. WANG[1], B. LIU[1], D. BARKER[1], L. MÓD[2], P. SZOCSICS[2], A. C. SILVA[3], Z. MAGLÓCZKY[2], M. MORALES[1];
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Hungarian Acad. of Sci., Budapest, Hungary; [3]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: Midbrain dopamine neurons have been documented in the nonhuman primate and human ventral tegmental area (VTA) and substantia nigra pars compacta (SNC) for nearly fifty years. Nonhuman primate studies have shown that midbrain dopamine neurons within these regions play key roles in reward, aversion, and learning. Clinical findings have led to the hypothesis that midbrain dopamine neurons are fundamental to addiction, schizophrenia, depression, and movement disorders. We have recently shown that glutamatergic neurons that express vesicular glutamate transporter 2 (VGluT2) are within subdivisions of the rodent VTA and SNC. Subsets of rodent VGluT2-expressing midbrain neurons co-express markers of dopamine or GABA synthesis, and are capable of co-transmitting glutamate with dopamine or GABA. Furthermore, our optogenetic studies in mice have shown that midbrain glutamatergic neurons participate in reward or aversion. However, the cellular compositions of the nonhuman primate and human VTA and SNC are unclear, contributing to a translational gap in our understanding of midbrain function. To determine whether glutamatergic neurons are present within the VTA or SNC of nonhuman primates and humans, we combined immunodetection of tyrosine hydroxylase (TH), and radioactive *in situ* hybridization to identify neuronal expression of transcripts encoding VGluT2 mRNA. In the marmoset VTA, half of rostral linear nucleus (RLi) neurons are VGluT2-only neurons and the other half are TH-only (dopaminergic). VGluT2-TH neurons are largely restricted to the parabrachial pigmented subdivision (PBP). The marmoset SNC pars lateralis subdivision contains VGluT2-only neurons but the SNC is otherwise dominated by TH-only neurons. Similar to the marmoset RLi, the human RLi contains equal percentages of VGluT2-only and TH-only neurons. However, a larger percentage of VGluT2-TH neurons are located in the human RLi (10%). Similar to the marmoset PBP, the human PBP contains the highest percentage of VGluT2-TH neurons (16%). The human SNC pars lateralis subdivision contains VGluT2-only neurons (16%) but the SNC is otherwise dominated by TH-only neurons. We conclude that subtypes of glutamatergic neurons are conserved from rodents to nonhuman primates and humans. Subtypes of glutamatergic neurons show preferential localization to discrete subdivisions, which are likely to participate in different circuits and functions. The discovery that glutamatergic neuronal subtypes are differentially distributed within the midbrain dopamine system from rodents to humans provides novel translational insight into studies of midbrain-related illnesses.

Disclosures: **D.H. Root:** None. **H. Wang:** None. **B. Liu:** None. **D. Barker:** None. **L. Mód:** None. **P. Szocsics:** None. **A.C. Silva:** None. **Z. Maglóczky:** None. **M. Morales:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 544.10/III34

Topic: G.02. Motivation

Support: SUNY BRAIN Network of Excellence Postdoctoral Fellowship

Title: Characterizing optically evoked dopamine in the olfactory tubercle of the rat brain using *In vivo* fast-scan cyclic voltammetry

Authors: *K. T. WAKABAYASHI[1], R. V. BHIMANI[2], C. E. BASS[3], J. PARK[1];
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Abstract: The olfactory tubercle (OT), as a component of the ventral striatum, serves as an important multisensory integration center for reward-related processes in the brain. Recent studies show that dense dopaminergic innervation from the ventral tegmental area (VTA) into the OT may play an outsized role in disorders such as psychostimulant addiction and disorders of motivation. However, due to its anatomical inaccessibility, relative small size, and proximity to other dopamine-rich structures, neurochemical assessments using conventional methods cannot be readily used. Recently, we investigated dopamine (DA) regulation in the OT of urethane-anesthetized rats using *in vivo* fast-scan voltammetry (FSCV) coupled with carbon-fiber microelectrodes, following optical stimulation of the VTA expressing channelrhodopsin-2 (ChR2), a non-native light sensitive cation channel, whose expression was driven by a generalized non-restricted promotor. Using FSCV, we compared DA modulation in the OT to the nucleus accumbens (NAc), a structure located adjacent to the OT and which also receives dense DA innervation from the VTA. We found that optical stimulation of DA occurred in two distinct regions of the ventral striatum, the OT and NAc. As well, optically evoked DA in the OT only occurred when the VTA was optically stimulated. Finally, we showed that DA transporters play an important role in regulating DA in the OT. However, the VTA is a highly heterogeneous brain region containing many different types of neurons including DA. Therefore the use of a generalized non-restricted promotor results in ubiquitous expression of ChR2 in all neurons in the VTA. Using a recently developed viral system, we have been able to target ChR2 expression to DA neurons in the VTA and are comparing the regulation of this neurotransmitter in different OT subregions.

Disclosures: **K.T. Wakabayashi:** None. **R.V. Bhimani:** None. **C.E. Bass:** None. **J. Park:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

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Program#/Poster#: 544.11/III35

Topic: G.02. Motivation

Support: NIH Intramural Research Program

Title: Dopamine D2 receptors on indirect pathway medium spiny neurons regulate motivation, but not learning, in a water T-maze

Authors: *T. J. O'NEAL[1], D. M. FRIEND[1], A. V. KRAVITZ[1,2];
[1]NIDDK, Bethesda, MD; [2]NIDA, Baltimore, MD

Abstract: Cognitive flexibility is the ability to adapt one's decision making based on current and prior circumstances. Within the striatum indirect pathway medium spiny neurons (iMSNs) have been implicated in cognitively flexibility and behavioral regulation, yet their exact role in cognitive flexibility has not been established. Here, we generated mice specifically lacking D2Rs in iMSNs (iMSN-D2R-KO mice) and tested learning and cognitive flexibility using a water T-maze reversal learning paradigm: mice placed at the base of a water-filled maze were taught to swim to an escape platform at the end of a single arm and, once learning criteria was reached, the platform location was reversed. iMSN-D2R-KO mice learned the location of the platform similarly to WT mice, as evidenced by similar number of days to reach criteria, errors within trials, and latency to reach the platform once initiating a trial. In addition, iMSN-D2R-KO mice reversed normally, as assayed with the same measures. However, iMSN-D2R-KO mice had a significant, progressively increasing delay to leave the entry arm at the start of each trial ($p < 0.001$) due to significant immobility within the entry zone ($p < 0.001$), and this delay became even more pronounced during reversal learning ($p < 0.0001$). These findings demonstrate that while iMSN-D2Rs are not necessary for either acquisition of the T-maze task or learning the new location of the platform during reversal, they appear to be involved in the motivation to initiate trials. We next tested iMSN-D2R-KO mice in a learned helplessness paradigm involving repeated forced swim tests and found that they did not differ from WT mice in the development or expression of learned helplessness, as measured by immobility within the water. We conclude that WT mice adapt their behavior from helpless to goal-seeking in the presence of an escape, as in the water T-maze, while iMSN-D2R-KO mice do not. This suggests that D2Rs on iMSNs are particularly relevant for motivating negatively reinforced behavior.

Disclosures: **T.J. O'Neal:** None. **D.M. Friend:** None. **A.V. Kravitz:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 544.12/III36

Topic: G.02. Motivation

Support: NIH Intramural Research Program

Title: Dorsal raphe nucleus serotonergic and glutamatergic neurons: distinct roles in reward learning

Authors: *R. A. MCDEVITT, R. M. MARINO, A. BONCI;
NIDA, Baltimore, MD

Abstract: The dorsal raphe nucleus (DRN) is a brain region best known for containing the majority of the brain's ascending serotonergic neurons. A subset of these serotonergic neurons express the vesicular glutamate transporter-3 (vGlut3) gene, which allows them to co-release glutamate. Additionally, the DRN contains a group of pure glutamate neurons that express vGlut3 in the absence of serotonergic markers. Recent studies suggest that mice will work to obtain optogenetic stimulation of serotonergic or glutamatergic DRN populations, although the relative strengths of these cell types in driving reward seeking have not been clearly defined. To address this, we have systematically compared self-stimulation rates of mice stimulating these populations. We directed expression of channelrhodopsin-2 (ChR2) to serotonergic or glutamatergic populations by injecting a cre-dependent viral vector (DIO-ChR2-eYFP) into DRN tissue of knock-in mice expressing cre recombinase controlled by the serotonin transporter (SERT-cre) or vGlut3 (vGlut3-cre). We allowed mice to perform a nose-poke task to obtain brief trains of laser stimulation, with light directed at DRN tissue. After several days of training, vGlut3-cre mice performed significantly more nose pokes than SERT-cre mice in this task, suggesting that the population of neurons primarily responsible for the reinforcing effects of DRN stimulation are non-serotonergic glutamate neurons. In future experiments we plan to explore the role of these neurons in reward learning tasks using optogenetic inhibition.

Disclosures: R.A. McDevitt: None. **R.M. Marino:** None. **A. Bonci:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

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Program#/Poster#: 544.13/III37

Topic: G.02. Motivation

Title: VTA projection neurons releasing glutamate and GABA in the dentate gyrus

Authors: *N. NTAMATI, C. LÜSCHER;
Dept. of Basic Neurosciences, Univ. of Geneva, Geneva, Switzerland

Abstract: Both dopamine and non-dopamine neurons from the VTA project to a variety of brain regions. Here we examine non-dopaminergic neurons in the mouse VTA that send long-range projections to the hippocampus. Using a combination of retrograde tracers, optogenetic tools, and electrophysiological recordings, we show that VTA GABAergic axons make synaptic contacts in the granule cell layer of the dentate gyrus, where we can elicit small postsynaptic currents (PSCs). Surprisingly, the currents displayed a partial sensitivity to both bicuculline and NBQX, suggesting that these meso-hippocampal neurons co-release both GABA and glutamate. Finally, we show that this projection is functional in vivo and its stimulation reduces granule cell-firing rates under anesthesia. Altogether, the present results describe a novel connection between GABA and glutamate co-releasing cells of the VTA and the dentate gyrus.

Disclosures: **N. Ntamati:** None. **C. Lüscher:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

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Topic: G.02. Motivation

Support: SNSF NCCR 'Synapsy'

ERC 'MeSSI'

Title: Resolving accumbal projections to lateral hypothalamus

Authors: *E. C. O'CONNOR, S. THOENI, C. LÜSCHER;
Dept. of Basic Neurosciences, Univ. of Geneva, Geneva, Switzerland

Abstract: Nucleus accumbens sends inhibitory projections that express dopamine D1Rs or D2Rs to several downstream targets, including ventral pallidum, lateral hypothalamus (LH) and ventral midbrain. Our previous work has shown that the dominant accumbal projection to lateral hypothalamus comprises dopamine D1R-expressing medium sized spiny neurons (D1-MSNs), which inhibit LH GABA neurons to rapidly control food consumption. Here using neuronal tracing, combined with *in vitro* electrophysiology in transgenic mouse lines that permit identification and control of specific cell types, we report that D1R-MSNs also inhibit LH glutamate neurons. We further explore the identity and projection specificity of these LH glutamate neurons and probe their role in food intake control and arousal. Taken together, our data suggest heterogeneity in accumbal projections to lateral hypothalamus and expand upon our understanding of ventral striatal output in the control of motivation and emotional behaviour.

Disclosures: E.C. O'Connor: None. **S. Thoeni:** None. **C. Lüscher:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

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Support: IWT Flanders

PF

IUAP

FWO flanders

HBP

Odysseus

Grants for Innovative Areas from MEXT, Japan

Title: Viral vector-mediated reversible blockage of the VTA-Nacc circuit and its role in motivational behavior in primates

Authors: *P. VANCRAEYENEST[1], J. ARSENAULT[1,2], K. ISA[3], K. KOBAYASHI[4], T. ISA[3,4,5], W. VANDUFFEL[1,2,6];
[1]Neuro and Psychophysiology, KU Leuven, Leuven, Belgium; [2]Massachusetts Gen. Hosp., Athinoula A. Martinos Ctr. for Biomed. Imaging, Charlestown, MA; [3]Dept. of Developmental Physiol., [4]Dept. of Viral Vector development, Natl. Inst. for Physiological Sci., Okazaki, Japan; [5]Dept. of Neuroscience, Grad. Sch. of Med., Kyoto Univ., Kyoto, Japan; [6]Radiology, Harvard Med. Sch., Boston, MA

Abstract: Although the role of the ventral tegmental area (VTA) in reward-driven behaviors is supported by electrophysiology (Schultz et al., 1997) and electrical microstimulation (Arsenault et al., 2014), the specific role of the VTA-Nucleus accumbens (NAcc) pathway in such behaviors remains untested in primates. We examined the neural and behavioral role of this specific circuit in macaques using a pathway-selective double-infection technique (DIT, Kinoshita et al. 2012). Neuronal transmission was blocked by injecting a highly efficient retrograde gene-transfer (HiRet) LVV in NAcc, followed by an injection of an AAV carrying a reverse tetracycline transactivator in VTA. Reversible expression of a fluorescent protein (FP)-tagged enhanced tetanus neurotoxin (eTeNT) in the VTA-to-NAcc projection neurons could be controlled by oral administration of doxycycline (DOX). To assess the effects of this intervention on functional connectivity and behavior, we used resting state fMRI (rfMRI) and a reward-based reversal discrimination paradigm. Afterwards, immunohistochemistry (IHC) and in-situ hybridization (ISH) was performed to determine the distribution and type of effectively inactivated VTA-NAcc projection neurons.
During the behavioral experiments, monkeys had to select between two simultaneously presented visual cues using a reward reversal paradigm. Reward probability varied across cue identity (1/3 vs. 2/3 of cue A and cue B trials were rewarded, respectively) which was reversed after a random number of trials. A learning model (Dayan & Abbot, 2001) applied to the data, revealed an increase in explorative behavior and decrease in learning rate after reversible inactivation of the VTA-NAcc pathway, bilaterally (N =2). The opposite effect was seen during DOX administration in naïve control monkeys (N = 2), indicating that ongoing learning in these monkeys was unaffected. Due to possible compensation of the contralateral hemisphere, behavioral effects during unilateral blockage were less pronounced (N =1). The rfMRI data showed a surprising but robust increase in functional connectivity between VTA and connected sites in all double-infected hemisphere (N=5), which suggests a release of inhibition of the VTA, possibly via a feedback loop, after inactivation of the VTA-NAcc pathway. IHC and ISH confirmed the successful double-transduction of both HiRet and AAV and therefore blockage of the VTA-NAcc neuronal circuit. Most of the FP-positive VTA neurons were TH-expressing dopaminergic cells. These results causally demonstrate the role of primate VTA-NAcc signaling in the regulation of stimulus-specific reinforcement.

Disclosures: P. Vancraeyenest: None. **J. Arsenault:** None. **K. Isa:** None. **K. Kobayashi:** None. **T. Isa:** None. **W. Vanduffel:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 544.16/III40

Topic: G.02. Motivation

Support: Medical Research Council

Title: Uncovering the molecular diversity of GABA neurons in the ventral tegmental area and substantia nigra.

Authors: *E. PAUL, K. TOSSELL, E. KALK, K. GREEN, J. LEIPER, M. UNGLESS; Neurophysiol., MRC Clin. Sci. Ctr., London, United Kingdom

Abstract: Midbrain dopamine neurons of the ventral tegmental area (VTA) and substantia nigra (SNC) are central to controlling voluntary movement, working memory, motivation and reward processing, as well as being implicated in multiple neuropsychiatric disorders. Dopamine neuron activity is strongly regulated by GABA neurons, including those found in the VTA and SNC, which make up around 30% of the neuronal population. Little is known about the functional roles of GABA neurons in this system and in particular whether there are functionally-distinct subgroups (e.g., dedicated interneurons, projection-specific output neurons and so on). Indeed, GABAergic neurons in other regions, including the hippocampus, cortex and spinal cord, exhibit considerable molecular, anatomical and functional diversity. We, therefore, hypothesized that GABA neurons in the VTA and SNC are also likely to exhibit similar levels of diversity. As a first step, we are seeking to uncover molecular markers that might identify distinct GABAergic subpopulations. To do this, we are taking two complimentary approaches. First, we are using a biased approach by investigating the expression of neuronal nitric oxide synthase (nNOS), parvalbumin and somatostatin, molecular markers known to identify GABAergic subgroups in other regions (e.g., hippocampus) using immunostaining. We find that nNOS is selectively expressed in a subset of GABA neurons in the parabrachial pigmented area of the VTA. In contrast, parvalbumin is expressed by a subset of dopamine neurons in the VTA; and somatostatin is absent in both the VTA and SNC. Second we are taking an unbiased approach, using tagged ribosomal affinity purification to isolate RNA specifically from GABA neurons in the ventral midbrain for RNA sequencing and the subsequent identification of novel molecular markers. Identification of these molecular markers, will allow for the future selective targeting and manipulation of subpopulations of GABA neurons in the VTA and SNC (by, for example, using cell-type-specific optogenetic stimulation) and a clearer understanding of the role of GABA neuron diversity within the midbrain dopamine system.

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Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 544.17/III41

Topic: G.02. Motivation

Support: DA029035

MH106972

Title: Investigating the role of mesolimbic dopamine transmission in incentive motivation

Authors: *B. HALBOUT[1], K. M. WASSUM[2], S. B. OSTLUND[1];
[1]Anesthesiol., Univ. of California, Irvine, Irvine, CA; [2]Dept. of Psychology, UCLA, Los Angeles, CA

Abstract: Cues that have been repeatedly paired with rewards can exert a strong influence over reward-seeking behaviors. In particular, such cues tend to acquire incentive motivational properties, which allow them to (1) trigger previously learned reward-seeking actions and (2) reinforce new actions. These sources of influence are separately studied using Pavlovian-to-instrumental transfer (PIT) and condition reinforcement tasks, respectively. While numerous studies suggest that mesolimbic dopamine transmission is critical for the acquisition and expression of incentive motivation, there is still debate regarding how dopamine contributes to this process, including whether phasic and tonic dopamine signals differentially contribute to this aspect of behavior.

Using in-vivo optogenetics, we investigated the effect of phasic activation of ventral tegmental area (VTA) dopaminergic neurons on the expression of cue-motivated reward seeking using a food-reinforced PIT task. Interestingly, our preliminary results indicate that such treatment has no impact on PIT performance, even though these stimulation parameters were effective in supporting intra VTA self-stimulation when delivered in a response-contingent manner. A separate experiment was conducted to examine whether an auditory cue paired with optogenetic VTA dopamine self-stimulation would develop the ability to conditioned reinforce. However, this study also found little evidence that phasic stimulation of mesolimbic dopamine release served as an incentive motivational signal. Together these results raise questions about the involvement of phasic dopamine transmission in incentive motivation.

Disclosures: **B. Halbout:** None. **K.M. Wassum:** None. **S.B. Ostlund:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

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Topic: G.02. Motivation

Support: MOST 103-2911-I-038-501 from the Ministry of Science and Technology, Taiwan

Title: Functional characterization of inputs between parabrachial nucleus and ventral tegmental area

Authors: *J.-H. TSOU[1,2], H.-J. YAU[1,3], A. BONCI[1,4,5];
[1]Cell. Neurobio. Res. Br., NIDA/NIH, Baltimore, MD; [2]Grad. Inst. of Med. Sciences, Col. of Medicine, Taipei Med. Univ., Taipei, Taiwan; [3]Grad. Inst. of Brain and Mind Sciences, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; [4]Solomon H. Snyder Dept. of Neuroscience, The Johns Hopkins Univ., Baltimore, MD; [5]Dept. of Psychiatry, The Johns Hopkins Univ., Baltimore, MD

Abstract: Taste helps establish food preference and the neural processing of taste aspect of food reward starts from the gustatory system. The gustatory information via cranial nerves reaches the brain first in the nucleus of the solitary tract (NST). The Parabrachial nucleus (PBN) receives the excitatory glutamatergic input from the NTS and has been shown to modulate feeding behaviors. In addition, PBN is also necessary to convey hedonic information of stimuli related to taste. Given that PBN sends substantial projections to midbrain dopamine neurons, it raises the possibility that taste stimuli may engage dopamine neurons in the ventral tegmental area (VTA) via PBN to modulate food intake. Thus, in the present study, we set out to study the functional connection between PBN and VTA in relationship to food consumption. We first carried out *in situ* hybridization to verify the neuronal types in PBN. In the past, locally electrical stimulation or pharmacological manipulation has the limitation to characterize defined connections. Thus, we would employ optogenetic approach by selectively express channelrhodopsin (ChR2) in specific Cre transgenic mouse line to target specific subtype of PBN neurons. We specifically tweaked PBN-to-VTA afferent input by delivering 473 nm blue light to the VTA via fiber optics while mice were performing behavioral task. The preliminary results showed that VTA receives functional inputs from the PBN and photostimulation of PBN-to-VTA input affects food consumption.

Disclosures: **J. Tsou:** None. **H. Yau:** None. **A. Bonci:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

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NIDA-INSERM Postdoctoral Drug Abuse Fellowship (VZ)

TRDRP 22FT-0063 (JHY)

Title: Ventral tegmental area glutamate neurons co-release GABA in a target-dependent manner to drive positive reinforcement

Authors: *V. ZELL, J. H. YOO, N. GUTIERREZ-REED, J. WU, R. RESSLER, M. A. SHENASA, A. B. JOHNSON, K. H. FIFE, L. FAGET, T. S. HNASKO;
Neurosciences, UCSD, San Diego, CA

Abstract: The ventral tegmental area (VTA) is a heterogeneous midbrain structure implicated in goal-directed behaviors. The VTA contains well-characterized dopamine neurons, but also GABA and glutamate neurons, subsets of which can co-release more than one of these transmitters. In our study, we report that optogenetic stimulation of VTA glutamate soma or terminals serves as a positive reinforcer on operant behavioral assays. Further, mice display marked preference for brief over sustained VTA glutamate neuron stimulation resulting in behavioral responses that are notably distinct when compared to dopamine neuron stimulation in operant choice and real-time place assays. Whole-cell recordings reveal EPSCs following stimulation of VTA glutamate terminals in the nucleus accumbens or local VTA collaterals; but reveal both excitatory and monosynaptic inhibitory GABA postsynaptic currents in the ventral pallidum (VP) and lateral habenula (LHb). The net excitatory effect in the VP and inhibitory effect in the LHb are both consistent with the measured relative GABA-glutamate components, and the observed rewarding behavioral effects. These data indicate that VTA glutamate neurons co-release GABA in a projection-target dependent manner and that their transient activation drives positive reinforcement distinct from dopamine.

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Poster

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Topic: G.02. Motivation

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NARSAD Young Investigator Award

Title: Ventral pallidum roles in cue-elicited sucrose seeking versus alcohol seeking

Authors: *J. M. RICHARD[1], N. STOUT[1], A. M. ARMSTRONG[1], P. H. JANAK[1,2];
[1]Dept. of Psychological and Brain Sci., [2]The Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: The mechanisms by which neutral cues elicit reward seeking are a critical area of inquiry in the neurobiology of addiction. Recently we have shown that activity of ventral pallidum (VP) neurons in response to a cue predicting reward availability encodes both the likelihood and latency of subsequent instrumental reward-seeking actions. Additionally, inactivation of VP neurons during the cue period reduces the likelihood and rapidity of reward-seeking behavior, suggesting that VP neurons encode and functionally contribute to the incentive motivational properties of cues. Here, we investigate whether VP neuron activity plays a similar role for conditioned responses to Pavlovian cues predicting reward delivery, and whether this differs for sugar versus alcohol rewards. Male and female Long Evans rats were trained to associate one auditory cue (the CS+) with delivery of 20% alcohol or 10% liquid sucrose reward and an alternative auditory cue (CS-) with no delivery of reward. Rats were trained until they entered the reward port on >70% of CS+ trials and <30% of CS- trials, and then were implanted with microwire arrays aimed at the VP. We find that ~25% of VP neurons (42/180) are excited by the CS+, and that these excitations are greater to the CS+ than the CS-, and greater on trials when rats make a port entry during the cue. VP neurons exhibit similar responses to cues predicting alcohol (10/21 neurons excited by the CS+). These results suggest that VP neural responses to Pavlovian cues may functionally contribute to cue-elicited reward seeking in the form of port entries. Consistent with this, in rats receiving sucrose, we find that pharmacological inactivation of VP with GABA agonists suppresses the number of entries and time spent in the reward port during the CS+, but not during the CS- or the pre-cue period. Further, in rats trained with alcohol, optogenetic inhibition via ArchT3.0 in VP neurons just during the cue period reduces the likelihood of a port entry and time spent in the port during the CS+, but not during the CS- (when port entry responses are already low). These results suggest that, in addition to

encoding and contributing to the ability of cues to invigorate instrumental reward-seeking actions, that VP neuronal activity during cue presentations is required for Pavlovian responses to reward cues for both natural and drug reward.

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Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

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NHMRC (Australia)

NSC Poland DEC-2012/05D/NZ4/02984

MSHE Poland 0020/DIA/2014/43

Title: Orexin and melanin-concentrating hormone (MCH) receptor activation in the nucleus incertus of adult rats alters locomotor activity and food intake

Authors: *A. SABETGHADAM[1], A. GRABOWIECKA[2], A. KANIA[2], A. BLASIAK[2], S. MA[1], A. L. GUNDLACH[1];
[1]The Florey Inst. Neurosci and Mental Hlth., Melbourne, Australia; [2]Dept Neurophysiol and Chronobiol, Jagiellonian Univ., Krakow, Poland

Abstract: The brainstem nucleus incertus (NI) contains a discrete population of GABA/peptide projection neurons that are highly responsive to psychological stressors via corticotrophin-releasing factor (CRF) and CRF1 receptor signaling [Ma S et al. J Physiol 591, 3981-4001, 2013]. Furthermore, the NI receives an orexinergic innervation from the lateral hypothalamus (LH) and expresses orexin receptors (OX1 and OX2), with strong activation observed via OX2 in vitro [Blasiak A et al. Neuropharmacology 99, 432-47, 2015]. Moreover, the NI relaxin-3 and LH orexin systems are implicated in the regulation of arousal and feeding [Ganella DE et al. Behav Pharmacol 23, 516-25, 2012]. As MCH and MCH1 receptor signaling is also involved in these processes and interacts with orexin signaling; with anatomical evidence of MCH1 expression within the NI; in the current study in adult rats, we examined the effect of acute bilateral injections into the NI of orexin-A and MCH and vehicle on locomotor activity and food

intake and the comparative effects of these peptides on NI neuron activity in vitro. Infusion of orexin-A (600 pmol) into the NI of conscious, satiated rats during the light phase produced an increase in total activity in a 240 min trial in a locomotor cell ($P<0.05$, n=5/group) and a modest increase in cumulative food intake ($P<0.01$, n=10/group) across a 4 h period, with increases seen from 30 min post-injection. Under the same conditions, intra-NI infusion of MCH (600 pmol) decreased the relatively low levels of locomotor activity ($P<0.05$, n=7-9/group) and the modest food intake observed in vehicle-treated rats ($P<0.05$, n=6/group). Interestingly, in 6 h light-phase, fasted rats, MCH (600 pmol) injected into NI during the early dark phase increased food intake for 1 h post-infusion ($P<0.01$, n=7/group), with no effect on the strong locomotor activity detected during the 240 min trial ($P>0.05$, n=6/group). Patch-clamp recordings revealed that ~1/3 of NI neurons tested were hyperpolarized by MCH (13/40 cells), with complex responses to MCH and orexin-A - 10 sensitive to orexin-A only, 6 MCH only, and 3 to both peptides. Other neurons tested (10) did not respond to either peptide. In conclusion, the impact of orexin/MCH inputs on NI neuron activity is largely consistent with those observed in other brain regions and related to modulation of integrated behavioral states such as sleep/wakefulness and appetite/metabolism. Further anatomical and functional studies are now required to determine how orexin/MCH projections to NI activate/inhibit different groups of neurons and circuits, and if this balance is altered during the light and dark phase, in relation to motivated activity and behaviors.

Disclosures: **A. Sabetghadam:** None. **A. Grabowiecka:** None. **A. Kania:** None. **A. Blasiak:** None. **S. Ma:** None. **A.L. Gundlach:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 544.22/III46

Topic: G.02. Motivation

Support: NIH grant NS076416

UW RRF grant to SJYM

NIA 5T32AG000057-39 grant to VT

Title: A novel role for the periaqueductal gray in consummatory behavior

Authors: *V. L. TRYON, S. J. Y. MIZUMORI;
Psychology, Univ. of Washington, Seattle, WA

Abstract: The periaqueductal gray (PAG) has a well-established role in pain processing, autonomic function, and behavioral responses to fear. However, anatomical work alludes to the PAG having a functional role in food intake and reward processing. Indeed, the PAG sends excitatory and inhibitory projections to both GABAergic and dopaminergic VTA neurons (Omelchenko & Seasack, 2010). In fact, the PAG provides the third heaviest subcortical glutamatergic input to the VTA (Geisler et al., 2007). Additionally, the PAG has been shown to have extensive reciprocal connections with a core feeding circuit in the brain (Betley et al., 2013). Therefore, our question was whether or not the PAG has a functional role in appetitive or consummatory behaviors. To address this, the PAG was reversibly inactivated during two separate food intake tasks in food-restricted male Long Evans rats. PAG inactivation resulted in a significant decrease in the amount of food consumed, as well as an increased latency to consume a palatable reward. Our next study investigated PAG neural responses to reward encounters as a different group of rats performed on a spatial working memory radial maze task. Rats were trained to retrieve large and small rewards (four or one 45 mg sucrose pellets) that were consistently found in the same locations on a radial eight arm maze. Once rats reached asymptotic performance levels on the task, they were implanted unilaterally with a 6-12 tetrode array aimed at the PAG. Analysis of the neural data reveals that in a subset of PAG neurons, there was phasic excitation at the onset of reward encounters as well as phasic inhibition in a separate subset of PAG neurons. These reward responses scaled with the size of the reward, with sustained excitation or inhibition in response to large rewards compared to small for reward-excited and reward-inhibited neurons. These results, taken together with the inactivation studies and anatomical evidence, suggest that the PAG may in fact play a role in consummatory behavior and reward related processing.

Disclosures: **V.L. Tryon:** None. **S.J.Y. Mizumori:** None.

Poster

544. Motivation Neurocircuitry: Brainstem Modulatory Centers

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 544.23/III47

Topic: G.02. Motivation

Title: Adaptor protein, X11 and X11L have distinct roles in exploratory activity.

Authors: *T. SATO[1], R. MOTODATE[1], Y. SANO[2], S. KAMADA[3], S. UCHIDA[3], T. SUZUKI[1];
[1]Hokkaido Univ., Sapporo-Shi, Japan; [2]Tokyo university of science, Chiba, Japan; [3]Kyushu Univ., Fukuoka, Japan

Abstract: X11/Mint1 (Munc18-1 interacting protein) and X11-like (X11L/Mint2) proteins are strongly expressed in the brain and evolutionary-conserved [1]. It is well known that X11 and X11L interact with many molecules such as Munc18-1, N-type calcium channels, β-neurexin, and hyperpolarization-activated cyclic nucleotide-gated potassium channel 2, and regulate synaptic function [1-6]. Though X11 and X11L are composed of similar functional domains such as a PTB (phosphotyrosine binding) and two PDZ (PSD95/discs large/ZO-1) domains, their expression pattern in the brain is so different. These findings indicate that they have different roles in the brain. So far, the involvement of X11L in the motivated behavior and cognition have been well analyzed [7]. However, the role of X11 in the higher brain function is not well studied, and also especially it lacks comprehensive comparison about their roles in the brain. To address this issue, we analyzed exploratory activity in the open field using X11-, X11L- and X11/X11L - double knockout (KO) mice. Interestingly, X11-KO mice and X11L-KO mice were opposite in the number of rearing and jumping. And the traveled distance was time-dependency increased in X11/X11L double KO mice, although it was time-dependency decreased in Wild Type mice. Our results indicate X11 and X11L have distinct roles in exploratory activity. 1) Rogelj, B. et al., Brain Res. Rev. 52, 305-315 (2006) 2) Okamoto, M. et al., J. Biol. Chem. 272, 31459-31464 (1997) 3) Maximov, A. et al., J. Biol. Chem. 274, 24453-24456 (1999) 4) Tomita, S. et al., J. Biol. Chem. 274, 2243-2254 (1999) 5) Biederer, T. et al., J. Biol. Chem. 275, 39803-39806 (2000) 6) Kimura, K. et al., Genes Cells 9, 631-640 (2004) 7) Sano, Y. et al., J. Neurosci. 29, 5884-5896 (2009)

Disclosures: **T. Sato:** None. **R. Motodate:** None. **Y. Sano:** None. **S. Kamada:** None. **S. Uchida:** None. **T. Suzuki:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 545.01/III48

Topic: G.03. Emotion

Title: A comparison of prefrontal cortex activity across emotional stroop and dot-probe tasks of attentional bias: a near-infrared spectroscopy study

Authors: ***K. J. KANGAS**, J. CARLSON;
Northern Michigan Univ., Marquette, MI

Abstract: Near-infrared spectroscopy (NIRS) research measuring prefrontal cortex (PFC) activity during emotional processing has been limited. Functional MRI research indicates that the amygdala and PFC are involved in orienting visuospatial attention to emotionally salient stimuli.

Two common tasks of attentional bias include the emotional Stroop and the dot-probe task. Both tasks appear to engage the PFC. However, research directly comparing attention-related PFC activation across these tasks is limited. Here, we examined PFC activity using NIRS while participants performed the dot-probe and emotional Stroop tasks in a counterbalanced order. In the dot-probe task participants had to locate a target dot, which was preceded by face pairs. There were three trial types: baseline (two neutral faces), congruent (dot appears behind the fearful face), and incongruent (dot appears behind the neutral face). The emotional Stroop task consisted of threating and neutral images surrounded by a boarder, which participants were asked to identify the color of. At a behavioral level, both tasks indicated that attention was captured by emotion. Reaction times were quicker for congruent (M = 361.07) compared to incongruent (M = 377.62) trials in the dot-probe task (t = -4.09, $p < 0.01$) and were slower for threatening (M = 615.13) compared to neutral (M = 603.30) images in the emotional Stroop task (t = 1.81, $p < 0.05$). At a neural level, both tasks elicited PFC activity. Thus by comparing these two tasks, NIRS data suggests the PFC is involved in emotional processing, along with orienting visuospatial attention to fearful stimuli.

Disclosures: K.J. Kangas: None. **J. Carlson:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

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Topic: G.03. Emotion

Support: GIST Research Institute (GRI) in 2016

Korea Research Institute of Standards and Science (KRISS)

Ministry of Culture, Sports and Tourism (MCST) and Korea Creative Content Agency (KOCCA) in the Culture Technology (CT) Research and Development Program 2015

Title: Difference with accordance and discordance of affective words with voice tone - A simultaneous EEG/MEG study

Authors: *M. KWON[1], H. CHO[1], S. AHN[1], K. KIM[2,3], S. JUN[1];
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Abstract: Emotion is evoked by various routes such as video clip, sound, smell, and touch. Among all, sound is a very important sense in that it may be delivered even when one's ears are almost closed and it may include significant information like emotion or intention from only one words. In this study, we investigated brain activation with collected EEG/MEG data when words are given with accordance or discordance of affective words with affective voice tone. Five positive and five negative affective words were spoken in Korean and each of words was vocalized with positive and negative voice tones. These stimuli consist of accordance and discordance set; accordance means positive (negative) words with positive (negative) tone, and discordance means positive (negative) words with negative (positive) tone. Twenty-three native Korean subjects (12 males, 11 females; age 23.82.9) listened the words and 21 EEG channels (Biosemi) and 152 MEG channels (KRISS, axial gradiometer system), vertical/horizontal EOG and EKG were measured simultaneously with 1024 Hz sampling rate. EEG and MEG data were filtered with 1 - 55 Hz, baseline corrected using 200 ms before stimulation, and EEG data were re-referenced with two earlobe channels. Power spectrum were calculated every 100 ms with time window of 100 ms. There was difference between accordance and discordance stimuli in the frontal area from EEG signal. When positive words vocalized with negative tone are given, the frontal area was more activated at 0 - 400 ms than those with positive tone. In addition, male subjects were more activated to accordance stimulus at 500 - 600 ms, where female subjects were not different. When negative words were given, accordance case was more activated, but male subjects lasted longer time (male: 100 - 700 ms, female: 200 - 300 ms). MEG showed the similar patterns to EEG, but notable different activation area between accordance and discordance was the temporal area, which yielded the shorter duration than EEG. From these results, it was believed that voice tone may be more influential on emotion than words, especially, negative tone may be critical. Furthermore, word processing is related with components at 400 - 600 ms, and brain activation of male subjects showed changes following meaning of words; thus it was inferred that the male may have more influenced with words than the female.

Disclosures: **M. Kwon:** None. **H. Cho:** None. **S. Ahn:** None. **K. Kim:** None. **S. Jun:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 545.03/III50

Topic: G.03. Emotion

Support: COI Stream Hiroshima

JSPS KAKENHI 26780418, 16H05958

Title: Extracting insular activation using scalp EEG during expectation of emotional picture.

Authors: ***N. KANAYAMA**, K. MAKITA, T. SASAOKA, M. MACHIZAWA, S. YAMAWAKI;
Hiroshima Univ., Hiroshima-Shi, Japan

Abstract: The insular cortex is a very important brain area for understanding human emotion. The anterior insular cortex as a subdivision of the insula has an important role for inference of interoceptive signals that underlie our emotional response [1]. Moreover, there has been growing interest in the insula as a hub for cognition and emotion [2]. However, the position of the insular cortex is within the lateral sulcus, which separates the temporal lobe and the inferior parietal lobe. The insular cortex is folded deep within the brain; therefore, it is difficult to measure the activation using scalp electroencephalogram (EEG). Here, we tried to extract the EEG activation of the insular cortex using independent component analysis (ICA) implemented in EEGLAB [3] and a new source estimation method implemented in SPM [4].
We conducted EEG and functional magnetic resonance imaging (fMRI) experiments separately with the same task: the emotional expectation and evaluation task using auditory cue (High: 4000 Hz, Middle: 1500 Hz, Low: 500 Hz), which indicates the valance of an upcoming picture, positive or negative. After the expectation period (4 sec), participants viewed a picture with emotional valance (4 sec). During the picture presentation period, participants were requested to evaluate the valance of the presented picture (very positive/ positive/ negative/ very negative). The presented pictures were selected from the International Affective Picture System (IAPS). We recorded scalp EEG during the emotional expectation and evaluation task and applied ICA on the data to exclude the external and biological noise. Using the scalp EEG data cleaned by ICA, the source estimation method with multiple sparse prior (MSP), implemented in SPM12 was applied. The activation maps, which were acquired by the fMRI experiment with an identical task, were used as a prior for source estimation. The activation map was obtained by one sample t-test for each condition (FWE, $p < .05$). As a result, we could successfully extract the activation of the insular cortex, which was significantly different for positive and negative expectations ($p < .05$, using permutation test). This significant difference was not observed by the same source estimation without the fMRI prior or ICA. This highlights the effectiveness of the combined use of ICA and MSP source estimation using fMRI prior on scalp EEG.
[1] Seth A K (2013). *Trends Cogn. Sci.*, 17, 565–73.
[2] Damasio A & Carvalho G B (2013). *Nat. Rev. Neurosci.*, 14, 143–52.
[3] Delorme A et al. (2011). *Comput. Intell. Neurosci.*, 2011, 130714.
[4] Litvak V et al. (2011). *Comput. Intell. Neurosci.*, 2011, 852961.

Disclosures: **N. Kanayama:** None. **K. Makita:** None. **T. Sasaoka:** None. **M. Machizawa:** None. **S. Yamawaki:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 545.04/III51

Topic: G.03. Emotion

Title: The utility of EEG frontal alpha power asymmetry in detecting emotional affect

Authors: *J. DREO, F. AGATIć, M. KAPITLER, Z. PIRTOŠEK;
Lab. For Cognitive Neurosci., Ljubljana, Slovenia

Abstract: It has previously been reported that asymmetries in frontal alpha-band power predict emotional affect in human subjects. While the limbic system is too deep to be reliably monitored using EEG, its widespread connections to the surface layers of the cortex offer an indirect way of inferring its activity. Basic electrophysiological investigations have confirmed a reliable inverse relationship between EEG alpha power and underlying cortical metabolic activity. Increases in alpha power thus correlate to decreased brain activity and vice versa. According to the frontal alpha power asymmetry hypothesis of affect detection, a relative increase of right vs. left frontal alpha power correlates to a subjective experience with positive emotional valence (approach) and a relative increase of left vs. right frontal alpha power with negative emotional valence (withdrawal). This method is increasingly being used in neuromarketing research to attempt to predict customer satisfaction with advertisements. We tested this hypothesis by conducting 128-channel EEG recordings on 22 subjects. The subjects were shown a series of 100 images from the Nencki affective picture system (NAPS). Half of the images were selected to have a strong negative emotional valence and half a strong positive one. The images were presented in a random sequence for 5 sec with an additional blank 5 sec “washout” period between them. EEG data was inspected and cleaned of artifacts (muscle, sweating, ocular) before being segmented into trials lasting from -1000 ms to +4000 ms relative to image onset. The average reference was calculated and spectral analyses (FFT and Wavelets) were performed on a channel by channel basis. Relative alpha power changes from baseline levels (before image onset) were calculated for each image. These were then averaged separately for positive and negative images. A statistical comparison was performed on several channel derivations and whole-scalp data. Additionally, the same analysis was run on data transformed with the spherical spline Laplacian operator. There were no statistically significant differences in left vs. right frontal alpha power asymmetry between negative and positive images. Our findings cast doubt on the reported robustness of a widely used method in neuromarketing research.

Disclosures: **J. Dreo:** None. **F. Agatić:** None. **M. Kapitler:** None. **Z. Pirtošek:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

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Topic: G.03. Emotion

Support: European Union's Seventh Framework Programme (FP7/2007e2013)/ERC Grant agreement number 295673

Title: The role of inferior parietal lobule and ventral premotor cortex in emotion body processing: a combined ctbs-fmri study

Authors: *T. ENGELEN[1], M. ZHAN[2], A. SACK[2], B. DE GELDER[2];
[1]Cognitive Neurosci., [2]Maastricht Univ., Maastricht, Netherlands

Abstract: The perception of emotion stimuli in the environment prepares our bodies for action, and especially when confronted with whole body expressions of an emotion like anger, action-related areas in the brain are triggered. Previously, parietal dorsal stream and premotor areas have been linked as crucial areas for processing of such stimuli, but questions about their contributions and specificity within the body processing network remain. We set out to disentangle the respective roles of dorsal stream areas crucial for action observation (Inferior Parietal Lobule (IPL)) and premotor areas involved in action preparation (ventral premotor cortex (PMv)) in the processing of emotional bodies by assessing local as well as remote effects of cTBS stimulation within the body processing network. Seventeen participants completed the experiment, which consisted of three fMRI sessions. Functional images were acquired using a 3T scanner with a 64-channel head-neck coil. During each session a passive viewing task was performed in which blocks of short videoclips of actors performing a neutral or angry action were presented. Two of the sessions were preceded by neuronavigated cTBS stimulation over either rIPL or rPMv based on individual anatomy. One session was used to assess baseline activity. During the baseline session two functional localizers were acquired to determine regions of interest (ROI). Functional ROIs include bilateral extrastriate body area (EBA), right temporoparietal junction (rTPJ), and right posterior superior temporal sulcus (rpSTS). Results indicate a general decreased response to both angry and neutral bodies in three of the functionally defined regions of interest (lEBA, rTPJ and rpSTS). Further analysis will focus on anatomically defined ROIs representing the areas stimulated (rIPL and rPMv), as well as whole brain group analysis comparing the three sessions and connectivity changes due to cTBS stimulation. Stimulation of IPL and PMv has previously shown effects on the behavioural level. The results of the current experiment extend these results by showing how also remote areas involved in body processing are affected by stimulation.

Disclosures: **T. Engelen:** None. **M. Zhan:** None. **A. Sack:** None. **B. de Gelder:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 545.06/JJJ1

Topic: G.03. Emotion

Title: Effects of obesity on levels of self-esteem in obese patients attending outpatiente of general hospital area family medicine unit no. 8 "dr. gilberto flores izquierdo" mexican institute of social security

Authors: *N. VEGA CABRERA[1], O. A. JARAMILLO-MORALES[2], E. VILCHIS-CHAPARRO[3], G. ESPINOZA-ANRUBIO[3], A. G. TORO-FONTANEL[3];
[1]IMSS , Medicina Farmiliar, UNAM, Mexico, Mexico; [2]CINVESTAV, Mexico City, Mexico; [3]IMSS, Mexico City, Mexico

Abstract: Background. Obesity can have physical or psychological consequences, within these last, we have emotional distress, loss of self-esteem and high levels of anxiety and depression .However, it is not well established whether obesity participates in the detriment of self-esteem. **Objective**. Determine the level of self-esteem in obese adult patients attending the outpatient of General Hospital Area Family Medicine Unit N° 8 in the Federal District. **Materials and methods**. Cross-sectional descriptive study. Inclusion criteria: age greater than 18 years, Mexican Institute of Social Security patients, regardless of gender or occupation. Sample: 234 patients. CI: 90%. Instrument: Coopersmith Self-Esteem Inventory (for adults). Dependent variable: levels of self-esteem and independent variable: obesity. **Results**. A ratio of 79.9% was obtained women and 20.1% men. With respect to self-esteem, a frequency of 7.3% low self-esteem, 14.5% average low self-esteem, high average 59.8% and 18.4% self-esteem high esteem in obese patients was found. Furthermore, it was shown that levels of self-esteem may increase or decrease depending on some factors including gender, age, education, type of obesity or marital status. **Conclusions.** The results show the first evidence indicating positive levels of self-esteem in obese adults attending the outpatient HGZ / UMF No. 8. Also, a positive or negative relationship in the self-esteem of obese patients which are mainly related to biological, economic, cultural and educational characteristics of each individual.

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Poster

545. Human Emotion: Social, Attention, and Other Influences

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Program#/Poster#: 545.07/JJJ2

Topic: G.03. Emotion

Support: Autism Science Foundation

NIMH Conte Center

Title: A late-positive potential originating from cingulate cortex represents the ambiguity of perceptual decisions

Authors: *S. SUN[1], S. WANG, 91125[2], S. ZHEN[6], Z. FU, 91125[3], D.-A. WU[4], S. SHIMOJO[5], R. ADOLPHS[2], R. YU[7];
[1]Humanities and Social Sci., Caltech, Pasadena, CA; [2]Humanities and Social Sci., [3]Dept. of Computing and Mathematical Sci., [4]Div. of Biol. and Biol. Engin., Caltech, PASADENA, CA; [5]Caltech, Division of Biology and Biological Engineering, CA; [6]Sch. of Psychology and Ctr. for Studies of Psychological Application, South China Normal Univ., Guangzhou, China; [7]Dept. of Psychology, Natl. Univ. of Singapore, Singapore, Singapore

Abstract: People often make decisions in the face of ambiguous information, but the neural substrates and response characteristics remain unclear. We used three types of ambiguous stimuli and combined EEG and fMRI to examine the neural representation of decisions about perceptual ambiguity. We identified a late positive potential, the LPP, which differentiated levels of ambiguity, and was specifically associated with behavioral judgments about choices that were ambiguous, rather than mere perception of ambiguous stimuli. Source modeling suggested that the LPP originated from multiple loci in cingulate cortex, a finding we further confirmed using fMRI and fMRI-guided ERP source prediction. Two further experiments revealed that the LPP encoded ambiguity in a domain-general fashion. Taken together, our findings argue for a role of an LPP originating from cingulate cortex in encoding decisions based on perceptual ambiguity, a process that may in turn influence others related to conflict and error correction.

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Poster

545. Human Emotion: Social, Attention, and Other Influences

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Program#/Poster#: 545.08/JJJ3

Topic: G.03. Emotion

Support: NYU Global Institute for Advanced Study

Title: Investigating the timecourse of aesthetic experience

Authors: *A. M. BELFI[1], E. A. VESSEL[3], D. G. PELLI[1], G. G. STARR[2];
[1]Psychology, [2]English, New York Univ., New York, NY; [3]Neurosci., Max Planck Inst. for Empirical Aesthetics, Frankfurt, Germany

Abstract: Aesthetic experience is an essential aspect of everyday life. Humans tend to seek out these experiences, such as listening to music, viewing paintings, and reading literature, which are often highly pleasurable. Such aesthetic experiences have been shown to modulate activity in several brain regions, which can be characterized, roughly, into three networks: sensory/motor, emotion/reward, and the default mode network. This suggests that aesthetic experiences are not the property of one brain region but may reflect dynamic interactions between large-scale brain networks. Here, we sought to investigate the dynamic nature of aesthetic experience using fMRI. To capture this inherently dynamic behavior, participants (N = 30) continuously rated their pleasure while viewing images of artworks. To further probe the time-variant nature of aesthetic experience, images were presented for different durations: 1s, 5s, or 15s. Each stimulus was followed by a 14s 'decay' period during which the participant continued to rate their pleasure even though the stimulus was no longer present. After this decay period, participants gave an overall summary rating of the artwork on a sliding scale. Trials were characterized as 'high,' 'medium,' or 'low' based on this overall rating. Stimuli consisted of 90 visual artworks presented in a randomized order. Artworks were museum-quality paintings drawn from various cultures (American, Asian, European) and time periods (from the 15th century to the present) and were selected to be unfamiliar to the participants. To investigate the neural dynamics during aesthetic experience, we performed ROI analyses investigating the timecourse of activity in sensory, emotion, and default mode networks. Our results indicate that presentation duration (1s, 5s, 15s) did not alter the overall proportion of images rated as high, medium, or low overall. Interestingly, this suggests that longer presentation time did not bias participants towards rating the images as more pleasurable. In addition, the overall summary ratings were highly correlated with peak values in the continuous ratings. Neuroimaging results indicated that non-visual brain networks showed activation peaks later in time from visual networks, and unlike visual networks, were sustained during the 14s decay period. These results suggest that aesthetic

experience is represented differently in a range of brain networks and that the timecourse of activity in these networks varies during aesthetic experience.

Disclosures: **A.M. Belfi:** None. **E.A. Vessel:** None. **D.G. Pelli:** None. **G.G. Starr:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

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Program#/Poster#: 545.09/JJJ4

Topic: G.03. Emotion

Support: Tsinghua University 985 Grant

Title: Representation of emotional contents in auditory stimuli by human insular cortex

Authors: Y. ZHANG[1], Y. DING[1], J. HUANG[2], W. ZHOU[1], Z. LIN[3], B. HONG[1], *X. WANG[4]; [1]Tsinghua Univ., Beijing, China; [2]Mind-Brain Inst., Johns Hopkins Univ., Baltimore, MD; [3]Dept. of Neurosurg., PLA Gen. Hosp., Beijing, China; [4]Dept Biomed Engin, Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: Accumulating evidence has suggested the role of the insula in emotion recognition. To examine whether the insula is involved in the perception of emotion from auditory stimuli, we recorded electrocorticographic (ECoG) signals from epilepsy patients when they were presented with emotional and non-emotional sounds. Neural signals recorded from different regions of insular cortex revealed different response properties. Posterior region of insular cortex, which is anatomically connected to sensory cortex, showed robust responses to all acoustic stimuli. Similar to the Heschl's gyrus, this region exhibited strong responses that are synchronized to the fundamental frequency of periodic stimuli. No differences were found in the posterior region of insular cortex between responses to emotional and non-emotional sounds. However, the anterior region of insular cortex on the right hemisphere showed robust high gamma responses to emotional sounds, but showed weak or no responses to non-emotional sounds, which indicated emotionally selective processing. In contrast, the anterior region of the left insular cortex did not show emotionally selective responses. Latency analyses showed much shorter response latency in the posterior region than the right anterior region of the insular cortex. Collectively, these observations suggest a role of the anterior insular cortex on the right hemisphere in transforming the basic sensory representations into inner affective representations.

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Poster

545. Human Emotion: Social, Attention, and Other Influences

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Program#/Poster#: 545.10/JJJ5

Topic: G.03. Emotion

Title: The relationship between emotion recognition performance and limbic gray matter volumes depends on sex and hormonal contraceptive use

Authors: *B. PLETZER[1], M. KRONBICHLER[1], J. CRONE[2], M. AICHHORN[1], H. KERSCHBAUM[1];
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Abstract: Emotion recognition is central to social interaction and is impaired in a variety of disorders associated with poor social functioning, e.g autism or schizophrenia. Several studies indicate that women display a higher accuracy in recognizing facial emotions than men, particularly for the recognition of sadness and fear. Neuroimaging studies demonstrate that emotion recognition is associated with the activation of limbic and para-limbic regions including the Amygdala, Hippocampus, Parahippocampus, Fusiform gyrus, Insula and cingulate gyrus, with different emotions activating distinct circuits. However, no study has previously related emotion recognition performance to brain structure and resting state functional connectivity. In the present study, 20 men and 53 women (20 naturally cycling, 17 taking androgenic contraceptives, 16 taking anti-androgenic contraceptives) underwent high resolution structural MRI as well as resting state functional MRI. They completed an emotion recognition task three times outside the scanner with two-week intervals between test sessions. The results demonstrate that emotion recognition performance relates to gray matter volumes in limbic areas. However, in men and users of anti-androgenic contraceptives larger gray matter volumes are associated with better performance, whereas in naturally cycling women and users of androgenic contraceptives larger gray matter volumes were associated with poorer performance. The strongest relationships between performance and gray matter volumes were detected for the recognition of disgust and sadness, however recognition of anger also related to gray matter volumes in men, while recognition of fear also related to gray matter volumes in women. Furthermore, emotion-recognition performance related to resting state functional connectivity with the limbic network in the left Insula across all participants. In summary, this study demonstrates for the first time that emotion recognition performance relies not only on the activation of limbic brain areas, but also on limbic brain structure and resting state functional connectivity. Moreover it demonstrates that the former relationship is modulated by sex of participants and hormonal contraceptive use.

Disclosures: **B. Pletzer:** None. **M. Kronbichler:** None. **J. Crone:** None. **M. Aichhorn:** None. **H. Kerschbaum:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 545.11/JJJ6

Topic: G.03. Emotion

Title: Do we always approach positive and avoid negative stimuli

Authors: ***C. FINI**[1], T. EVEREART[2], L. BARDI[2], M. BRASS[2], A. MOORS[2];
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Abstract: Research supports that positive stimuli lead to approach and negative stimuli to avoidance. To date, no studies have investigated this approach-avoidance effect using transcranial magnetic stimulation (TMS). Twenty students took part to this experiment. In the practice phase, they learned to press a button with the index finger to approach a stimulus and to press a button with the thumb to avoid the stimulus, depending on verbal instructions. When choosing "approach", a manikin moved toward a dot in the center of the screen; when choosing "avoid" the manikin moved away from the dot. In the experimental phase, the dot was replaced with images with a positive or a negative valence. In the compatible block, participants were instructed to approach positive and avoid negative stimuli. In the incompatible block, the instructions were to avoid positive stimuli and approach negative stimuli. Participants could respond only after hearing the instruction "choose". A TMS pulse was given 400 ms post stimulus onset, and MEPs from the first dorsal interosseous (FDI) and the opponent pollicis (OP) of the right hand were registered. The experiment was composed of two sessions: in the first session participants approached with the index finger and avoided with the thumb; in the second session the action-finger mapping was the opposite. The results showed that only the MEPS registered at the FDI were significantly modulated by the instruction, so the analyses were restricted to this finger. When approaching, the motor readiness was modulated by the instructions. Specifically, in the compatible blocks MEPs were significantly larger for positive than for negative stimuli; in the incompatible blocks, MEPs were significantly larger for negative than positive stimuli. When avoiding, instructions did not modulate MEPs amplitude. Indeed, MEPs were significantly larger with negative than positive stimuli irrespective of instructions. Thus approaching seems to be a more flexible action tendency than avoidance, since participants

can equally follow the instruction to approach positive and negative stimuli, whereas they can more easily follow the instruction to avoid negative compared to positive stimuli.

Disclosures: C. Fini: None. **T. Evereart:** None. **L. Bardi:** None. **M. Brass:** None. **A. Moors:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 545.12/JJJ7

Topic: G.03. Emotion

Support: NIMH-DIRP

Title: Emotion processing in moebius syndrome patients

Authors: *S. LOKEY, S. JAPEE, C. BAKER, L. UNGERLEIDER;
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Abstract: Moebius Syndrome (MoS), a rare congenital neurological disorder, is characterized by abnormality of the VIth and VIIth cranial nerves, resulting in paralysis of the face and a lack of skeletal muscle feedback. The facial feedback hypothesis, a prominent concept in face perception, asserts that feedback from the skeletal muscle of the face alters the experience of emotion (Buck, 1980; Soussignan, 2001; Strack, Martin, & Stepper, 1988). Thus, it is possible that individuals with MoS have trouble identifying or processing emotion. Several studies have examined the ability of individuals with MoS to recognize familiar and unfamiliar faces (Calder et al., 2000; Bate et al., 2013), to experience and express emotion (Cole, 2010), and to recognize and label facial expressions (Giannini et al, 1984; Calder et al, 2000; Bogart & Matsumoto, 2010; Bate et. al, 2013). However, results from these studies have been mixed and it is thus unclear whether MoS leads to deficits in emotion processing. One reason for the conflicting results could be that most of the studies used only an emotion-labeling task, which may not have been sensitive to subtle deficits in emotion processing. Thus in our study, in addition to an emotion-labeling task, we used an emotion-detection task to assess emotion processing in individuals with MoS. Participants were shown morphs of neutral to fearful and neutral to happy faces, and were instructed to distinguish between fearful and neutral, and happy and neutral faces, with a button press. A one-up, three-down staircasing procedure was used to determine each participant's threshold for 79% accuracy. The same morph stimuli and staircasing procedure were used in a feature-detection control task, where participants were instructed to indicate with a button press whether the face depicted an open or closed mouth. For the emotion-

labeling task, participants were shown faces depicting each of 7 emotions (happy, sad, anger, disgust, fear, surprise, neutral) and instructed to press a button corresponding to each emotion. Analysis of threshold levels for the emotion-detection task revealed that individuals with MoS, as compared to controls, showed a deficit in detecting emotion, more so for fearful than happy faces. Performance on the emotion-labeling and control tasks did not differ between individuals with MoS and controls. These results indicate that the paralysis of, and lack of feedback from, the facial muscles in MoS may indeed impair emotion perception, but that this impairment is more in the ability to detect emotion rather than identify it.

Disclosures: S. Lokey: None. **S. Japee:** None. **C. Baker:** None. **L. Ungerleider:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 545.13/JJJ8

Topic: G.03. Emotion

Title: Emotional evaluation between young and middle adults with the International Affective Picture System

Authors: *X. CORTIJO-PALACIOS[1], E. ACOSTA-MARI[2], B. BERNAL-MORALES[3], C. GUTIERREZ-GARCIA[4], T. CIBRIAN-LLANDERAL[5];
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Abstract: Introduction: the International Affective Picture System (IAPS) uses a series of emotional, normative and internationally accessible pictorial stimuli and is considered to be the most reliable and valid system in the experimental study of emotions (Lang et al., 2008). Nevertheless, several studies have been conducted in a population group with very defined characteristics (college students and young adults) so the question arose whether emotional evaluation differ if the images are analyzed by a group of middle adults.
Objective: the purpose of this study was to examine if there are differences in the assesment of one block of sixty images from the IAPS database between young and middle adults.
Participants and Methods: in order to examine differences in the evaluation of IAPS images, we had two groups. The first group was composed for thirty-seven young adults (YA; 46% female and 54% male; mean age 20) and the second group was a sample of fifty middle age (MA; 66% female and 34% male; mean age 49), both groups participated voluntarily. Pictures task: the

pictures were selected from the fifteen block of IAPS database (Lang et al., 2008). The 60 images were set in a power-point presentation and presented individually during 5 sec followed by 5 sec of transition between images. Emotional response was recorded immediately after watching each image using the Self-Assessment Manikin (SAM) 9-point scale for registering the dimension of valence (pleasure) proposed by Bradley and Lang in 1994.
Preliminary results: we found significant differences between young and middle adults in 46% of the slide numbered pictures evaluated: 2153 (p = .0005); 2306 (p= .01902); 2332 (p= .0065); 2358 (p=.0130); 2594 (p=.0388); 2598 (p=.0050); 2704 (p=.0003); 2718 (.0022); 2799 (p=.0195); 2811 (p=.0451); 3017 (p=.0128); 3191 (p=.0026); 3225 (p=.0380); 4006 (p=.0049); 4225 (.0485); 4559 (p=.0347); 5961 (.0005); 6021 (p=.0485); 6825 (.0071); 7056 (p=.0498); 8205 (.0273); 9254 (p=.0010); 9425 (p=.0001), 9426 (.0052); 9427 (p=.0003); 9428 (p=.0177); 9900 (p=.0001), 9901 (p=.0002) t-test.
Conclusions: The findings demonstrate differential processing of emotional content between young ang middle aged adults and may suggest that the human response to affective pictures depends on the development stage and cognitive abilities. Currently we are evaluating the emotional discrimination between different population considering age, gender, education level and chronic diseases like cancer.

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Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 545.14/JJJ9

Topic: G.03. Emotion

Title: The emotional valence of subliminal priming affects perception of facial expressions

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Abstract: Objectives: We conducted a psychophysical study to determine whether the emotional content of priming images presented subliminally (so briefly that they could not be consciously perceived) affected the subsequent perception of facial expressions.
Methods: The experiment consisted of a priming image shown for 16 ms, followed by a 100ms mask which was a scrambled version of the priming image, followed by a 500 ms presentation of an unfamiliar face (test-face) showing an angry or neutral expression. Observers indicated via

keypress their perception of the test-face expression.
Data was analyzed using a GLMM and an unpaired t-test to characterize the relationship between performance on the test-faces and the content of the primes: presence/absence of a face, centricity (egocentric, directed at the observer, or allocentric, directed away from the observer), and affective category of the prime (aggressive, pleasant, neutral). All prime images were selected from the IAPS [1] database and from image-data bases on the web, and were processed in Matlab to normalize for luminance and size.
Results: Emotional valence of priming images affected perception of test-face expressions. The effect was statistically significant only for aggressive and pleasant primes. Performance on neutral test-faces was significantly worse than on angry test-faces for the aggressive primes which contained a face. In trials displaying pleasant priming images, performance on angry test faces was significantly poorer than on neutral-test faces. Also, performance on angry test-faces was significantly poorer for egocentric primes (whether they contained a face or not).
Conclusion: Perception of target-face expressions was selectively affected by the emotional content of the affective primes. The valence of the priming images biased observers to categorize target-faces in an emotionally congruent way with the affective valence of the prime, although they could not consciously perceive them. All egocentric aggressive primes significantly biased perception of test-faces as angry, even when they showed a neutral expression. These results suggest that emotional valence, although not consciously perceived, can significantly influence conscious perception of emotional expressions.
[1] Lang PJ et al (2005). *International affective picture system (IAPS)*: NIMH, Center for the Study of Emotion & Attention.

Disclosures: **M.A. Huang:** None. **K.D. Rana:** None. **L.M. Vaina:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 545.15/JJJ10

Topic: G.03. Emotion

Support: MITACS

Title: Lifestyle factors associated with performance in mobile affective training tasks

Authors: ***C. H. LIN**[1], K. YIP[2], M. BAXTER[2], C. RANKIN[1], P. NUSSBAUM[3];
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Abstract: Lifestyle and demographic factors such as age, gender, education, health condition, physical activity, cognitive activity, social relationships, and nutrition may associate with performances in affective tasks. We compared the affective training performance of over 1 million users from the Fit Brains Trainer by Rosetta Stone with answers to 99 factors collected from the Brain Health Lifestyle® Assessment. We found that age and gender have an effect on performance and the strategy used to solve emotion stroop (ES) and facial expression recognition (FE) questions. Education had some protective effect on the age-dependent decline in performance, and had a different effect on gender-dependent differences in problem-solving strategy. Some factors were associated with performance in both ES and FE tasks, such as having regular social discussions, regular travel, marital status, and playing musical instruments. Other factors were better associated with performance in ES tasks (i.e. searching the Internet daily, artistic or creative activity), or in FE tasks (i.e. regular meditation and mindfulness practices, having depression or anxiety). This study provided insights on the relationship between lifestyle choices, psychosocial conditions, and the performance in emotion/affective tasks for a population that actively participated in mobile brain training.

Disclosures: **C.H. Lin:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; MITACS/Rosetta Stone. **K. Yip:** A. Employment/Salary (full or part-time): Rosetta Stone. **M. Baxter:** A. Employment/Salary (full or part-time): Rosetta Stone. **C. Rankin:** None. **P. Nussbaum:** F. Consulting Fees (e.g., advisory boards); Rosetta Stone.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

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Topic: G.03. Emotion

Support: Private gift

Title: Measuring mother-child emotional connection via the welch emotional connection scale (wecs)

Authors: ***M. G. WELCH**[1], A. A. HANE[2], J. N. LACOURSIERE[3], R. J. O'DRISCOLL[3], R. J. LUDWIG[3], M. M. MYERS[4];
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Abstract: We hypothesize that a child's biological regulatory mechanisms are dependent on reciprocal emotional connection and its accompanying physiological co-regulation. When the mother and child are positively co-regulated they approach each other more often; show mutual interest in each other; and exhibit eye contact, and responsiveness to each other's touch and affective state. The more this co-regulation occurs, the stronger the emotional connection becomes. Thus, physiological co-regulation and emotional connection, which we posit are the basis of healthy human development, are novel treatment targets. Most current interventions for emotional behavioral developmental disorders (EBDs) focus on helping children, even infants, to self-regulate. Due to this emphasis, current treatment outcome measures assess parameters of self-regulation of the child or potential of the mother to help the child self-regulate. Instead, we sought a scale that would measure co-regulation and emotional connection. The need for such a new measure emerged from Family Nurture Intervention (FNI), which is based on establishing mother-infant co-regulation and emotional connection. FNI is a novel approach to the treatment of children age 0-5 with EBDs. FNI is experiential, not didactic, guided by a Nurture Specialist who engages mother and child in repeated calming sessions to condition their visceral/autonomic nervous systems to respond to contact with each other by calming. A calm state is required for optimal learning and relating. As there is no instrument for assessing emotional connection, we have developed a new scale, the Welch Emotional Connection Scale (WECS). The WECS is designed to measure emotional connection through observable elements of mother-child interaction, scored either live or on video, for use in clinical and research settings. It has four constructs on which mother and infant are scored; attraction to each other, verbal communication, facial expressiveness, and mutual sensitivity/responsivity. In addition there is a binary (yes/no) determination to assess the mother and child's emotional connection. Preliminary results show high inter-rater reliability and construct validity. The WECS is being correlated with heart rate variability during mother-child interactions. The WECS is proposed as a tool to assess interventional need as well as treatment efficacy. The brevity of the scale is designed for quick and effective assessment in clinical settings. More importantly, the existence and implementation of this measure will shift the field toward targeting physiological co-regulation and emotional connection, no matter what the intervention.

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Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

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Topic: G.03. Emotion

Support: MOE AcRF Tier 1 (H.X.)

NTU Research Scholarship (C.L.)

School of Humanities and Social Sciences Postdoctoral Fellowship (E.B.)

SRG2013-00035-FHS (Z. Y.)

Title: Neural correlates of facial emotion adaptation and autistic traits

Authors: ***H. XU**[1], C. LUO[1], X. LIN[2], E. BURNS[1], X. WANG[3], Z. YUAN[2];
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Abstract: Autistic subjects have impaired recognition of facial emotions and face identity. However, the neural mechanism of the impaired facial emotion recognition is not fully understood. In the current study, we investigated subjects' performance in facial expression adaptation on partially occluded faces, by manipulating the flickering patterns of occluding bars. Three sets of adapting faces were generated by manipulating the synchronization of flickering facial parts (e.g., eyes and mouth), e.g., coherent, partially or incoherent flickering. Test faces are full faces ranging from sad to happy. After adapting to the flickering faces, subjects' task is to judge whether the test face is a happy or sad face. Baseline condition without adaptation is also included. Subjects showed significant facial expression aftereffect when the adapting face was perceived as flickering coherently, but relatively weaker in the disrupted conditions (partial and incoherent flickering). The flickering patterns in the adaptors also modulate the amplitude of both the early component (N170) and late components (~400ms) of ERPs for the test faces. Moreover, we observed significant positive correlation between the amplitude change in these components with the AQ scores of the subjects. As the early component is suggested to indicate the response to the onset of the face stimuli, and late component indicates the processing of meaning, our results indicate that both local and holistic processes are critical for facial emotion perception, and the integration between local and holistic processes is related to the autistic traits of the subjects.

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Poster

545. Human Emotion: Social, Attention, and Other Influences

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Program#/Poster#: 545.18/JJJ13

Topic: G.03. Emotion

Support: NIH R01 HD0691780

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F31 NIMH 107119

Title: Social influence shapes neural and behavioral responses to emotional scenes.

Authors: *R. E. MARTIN, J. WEBER, K. N. OCHSNER;
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Abstract: Emotions, though generated individually, are strongly influenced by other people. The goal of this study was to examine how other people shape our emotions. We scanned participants while they viewed and rated how pictures of negative and neutral social scenes made them feel. Following each rating participants were shown what they believed to be a group rating from a sample of approximately 100 peers. After a rest period, participants then rerated the same scenes a second time, this time without seeing peer ratings. We found a strong main effect of social influence such that participants changed their second ratings of the scenes to conform to those of their peers. At the neural level, our findings replicated previous studies (e.g., Klucharev et al., 2009; Izuma & Adolphs, 2013) demonstrating activation of the dorsomedial prefrontal cortex during peer conflict (when peers rated items higher or lower than participants). Building off these prior studies, we found that magnitude of activation within subjects predicted greater conformity to peer influence. We also found activity in lateral prefrontal regions commonly associated with emotion regulation during peer conflict. Additionally, we found that amygdala activation increased during the second rating period when peers rated those scenes as more negative. Taken together these findings suggest that social influence modulates neural circuitry associated with emotional responding.

Disclosures: R.E. Martin: None. **J. Weber:** None. **K.N. Ochsner:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

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Program#/Poster#: 545.19/JJJ14

Topic: G.03. Emotion

Title: Human social brain in media reception: the effect of emotional valence of video stimuli

Authors: *S. TUKAIEV[1], I. ZYMA[1], A. VASILCHENKO[1], D. KASHPUR[3], Y. HAVRYLETS[4], V. RIZUN[4], M. MAKARCHUK[2];
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Abstract: Modern society increasingly depends on mass media, which may be accompanied by negative changes in mental health. TV is considered to be the most effective mass media channel due to the large audience and its influence on the psychophysiological and emotional condition of the viewers. The aim of this study was to determine the structure of audience ratings and the interrelation of negative TV news plots and ads perception. 86 healthy volunteers (women and men) aged 17 to 26 years participated in this study. We recreated the conditions similar to the "natural" TV news program and offered 4 TV-news plots (each 1-1,5 minutes long, containing violent episodes), interrupted by a pause for three 30 seconds-long ads (food and drink). EEG was registered over a period of 3 minutes during the rest state, 10 minutes during watching 5 TV plots, and 6 minutes of aftereffect. We estimated the spectral power density of all frequencies from 0.2 to 45 Hz. The Wilcoxon test was carried out to compare dependent samples data. After watching the news and the ads, the participants were asked to recall and evaluate them. The plots and the ads were evaluated as follows: on the scale "unpleasant - pleasant", the news stories were perceived as rather unpleasant, the ads – as rather pleasant; on the scale "relaxing - activating", the negative plots were perceived as rather activating, the ads – as rather relaxing. Neurodynamics showed that viewing both TV news stories and TV commercials caused activation changes in the information-analytical cognitive processes of neural networks. They increased actualization of attention (depression alpha2 rhythm), short-term memory with an emotional component (increase in theta1,2 in the central-posterior and right frontal areas only for negative TV news), as well as semantic-cognitive and emotional processes (depression alpha3 and exaltation of beta1,2 bands). Increasing the number of viewed TV news plots with ads interruption in spite of their negative emotional content led to the development of intellectual processes of adaptation (no changes in the reactivity of the theta-rhythm and activity reduction of cognitive beta1,2 and alpha3 neural networks). Depression of alpha1,2-bands (external attentional system) demonstrates the activity of the descending control systems. We revealed the absence of such changes in the functional activity of brain under prolonged viewing of TV ads. We demonstrated inhibitory effect of ads viewing on the activation of cognitive neural networks in response to watching TV news despite their negative emotional orientation. This suggests that viewing TV advertising and TV news are active cognitive processes.

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Poster

545. Human Emotion: Social, Attention, and Other Influences

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Program#/Poster#: 545.20/JJJ15

Topic: G.03. Emotion

Title: Neural correlates of acceptance and rejection during online interaction in teenagers.

Authors: *I. PERINI, P. GUSTAFSSON, P. HAMILTON, R. KÄMPE, M. ZETTERQVIST, M. ZETTERQVIST, M. HEILIG;
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Abstract: Negative online social interactions incl. cyberbullying are a growing problem among teens. Here, we investigated the neural correlates of social interaction in a simulated online context. Healthy teenagers (age 15 to 18) engaged in a social online game in which they chose whether they liked or disliked pictures of other young adults. Similarly, their own pictures were judged by others. A picture of a young adult was shown on the screen for 3 seconds, and the subject was instructed to choose whether he or she liked that person or not by pressing a button. Following this choice, a “thumbs up” or a “thumbs down” appeared on the left side of the picture as feedback. The word “calculating” was then displayed over the picture for 2-4 sec, and signaled that other people were also rating the person (anticipation phase). Finally, the collective outcome was superimposed over the picture and shown for 3 seconds (outcome phase). Similarly, participants saw their own picture being rated by others. There were 16 “self” and 16 “other” trials occurring in two consecutive runs in the MR scanner. A within subject t-test for the anticipation and outcome phases and a 2x2 factorial ANOVA with factors Perspective (self and other) and Outcome (like and dislike) for the outcome phase were performed. During anticipation, right anterior insula (rAI) was significantly more active for self versus other conditions. During the outcome phase, rAI and the head of the right caudate were significantly more activated when the participant saw themselves as opposed to other people being liked. Significant activations were also found for the main effect (ME) of Perspective and the ME of Outcome. For the ME of Outcome left insula, posterior cingulate cortex (PCC) and dorso-medial prefrontal cortex (dmPFC) were activated. Beta values were higher in insula for the level “like” whereas dmPFC and PCC showed higher values for the level “dislike”. For the ME of Perspective, rAI and mid cingulate cortex (MCC) were significantly more active for the condition “self” versus “other.” These findings show that areas implicated in salience and goal directed behavior are more engaged when the subject is being judged. This paradigm offers insights about neural substrates of online communication during adolescence and can be used to investigate social interaction in individuals with more vulnerable psychological profiles.

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Poster

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Topic: G.03. Emotion

Support: NRF Thuthuka - TTK1206211523

Title: The impact of mathematics anxiety on mathematics performance at pre- and university level

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Abstract: Aim: Mathematics anxiety (MA), a state of discomfort associated with performing mathematical tasks is thought to have a negative impact on pre-university students. The impact of MA on mathematics performance by university students is not fully understood. The aim of the current study was to establish the impact of MA on mental and advanced mathematics problem solving by pre- and university students while controlling for test anxiety (TA), a construct related to MA but which is typically not controlled for in most MA studies. **Methods**: Two-hundred and fifty students who have just obtained their matriculation (matric) and have been granted admission to study at the university participated in the study. They (1) completed a questionnaire containing the AMARS (Abreviated Mathematics Anxiety Rating Scale) and Sarason's Test Anxiety Scale, (2) performed simple addition and multiplication mentally and (3) participated in first year university mathematics evaluations. Their matric mathematics performance together with data from 1 - 3 was analyzed. **Results**: Mathematics performance differences as a function of mathematics anxiety was revealed in both mental and advanced mathematics at pre- and university levels. No gender differences emerged for mathematics performance but levels of MA and TA were higher for female than for men. A positive correlation between MA and TA with a negative correlation between MA and mathematics performance was obtained. TA was also negatively correlated with mathematics performance. **Conclusions**: Our study shows that performance in university mathematics is negatively affected by MA experience from secondary/high school. Preoccupation with math fears and anxieties might have function like a resource-demanding secondary task during mathematics solving by high math anxious students. Girls showed higher levels of MA than boys and high levels of MA

were related to poorer levels of mathematics performance. Therefore MA warrants attention in the mathematics classroom both at secondary and university level. In fact the cognitive literature now shows how critically math performance depends on working memory, for any form of arithmetic and math that involves processes beyond simple memory retrieval. **Key words**: Mathematics anxiety and test anxiety

Disclosures: E.T. Muluh: None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 545.22/JJJ17

Topic: G.03. Emotion

Title: Overprotection with the familial environment and fMRI responses to emotional context: Exploratory evidence.

Authors: *P. PANDHER[1], V. DIWADKAR[2], K. RAMASESHAN[2], M. RE[3], P. BRAMBILLA[4], M. NOBILE[5];
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Abstract: Overprotection is defined as the degree of parental control within a familial environment, and parental overprotection mediates development of emotional responses (Parker et al. 1979; Ingram et al. 2001), though the neuronal correlates of these effects have remained unexplored. Here we explored the effect of parental overprotection estimated using the parental bonding instrument (PBI; Parker, 1989) on brain responses when subjects committed attention to negative, positive or neutral valenced faces (a proxy for assessing emotional responses).
Method:
Thirty-one typically developing subjects (N=31, =15.4) participated in an affective attention task where rapidly presented faces of varying valence were presented in blocks (30 s). To contextualize responses based on affective context (signaled before each block) faces had an “X” or “A” marked on the bridge of the nose. An “X” was a target only if the face was consistent with the block’s affective context. fMRI data (3T Philips Achieva) were processed in SPM8 with typical methods. First level contrasts were employed to identify fMRI correlates of activation to negatively, relative to positively valenced context. These maps were submitted to second level regression models wherein maternal and paternal overprotection scores from the PBI were modeled as covariates of interest. Significant clusters were thresholded ($p<.05$, cluster).

Results:
An *increase* in both maternal and paternal overprotection predicted *decreases* in fMRI responses to negative affective context. These effects were observed in specific regions of *both* maternal and paternal scores including the Cerebellum, Middle Frontal Gyrus, Fusiform Gyrus, Lingual Gyrus, and Middle Temporal Gyrus. Specific effects for each parent were also observed in the Superior Frontal Gyrus, Middle Occipital Gyrus, Middle Temporal Gyrus, and Lateral Occipitotemporal Gyrus (maternal), and in the Medial Frontal Gyrus, Anterior Cingulate, Inferior Parietal Lobule, and Insula (paternal).
Discussion:
Social and emotional development in an overprotective familial environment can adversely affect future interpersonal relationships and present vulnerability for psychiatric conditions (Ingram, et al. 2001). Our exploratory analyses suggest that parental overprotection statistically exerts inhibitory effects on fMRI responses to negatively emotional context. Though the specific mechanisms are unclear, it is plausible that these decreases particularly in cognitive and emotional control regions such as the cingulate and prefrontal cortices reflect signatures of controlling parental style on emotional regulatory responses.

Disclosures: P. Pandher: None. **V. Diwadkar:** None. **K. Ramaseshan:** None. **M. Re:** None. **P. Brambilla:** None. **M. Nobile:** None.

Poster

545. Human Emotion: Social, Attention, and Other Influences

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 545.23/JJJ18

Topic: G.03. Emotion

Support: DARPA

Title: Neurophysiological indices of human - robot interactions.

Authors: *S. J. SMITH[1], B. T. STONE[1], T. RANATUNGA[2], A. MANNA[3], T. Z. RAMSOY[4], C. BERKA, 92008[5];
[1]RESEARCH, ADVANCED BRAIN MONITORING, Carlsbad, CA; [2]Fello Robots, San Jose, CA; [3]Lowe's Innovation Lab., Charlotte, NC; [4]Neurons Inc., Holbaek, Denmark; [5]Advanced Brain Monitoring, CARLSBAD, CA

Abstract: Human Robot Interaction (HRI) has long been a subject of interest, attracting scientists and engineers to explore possibilities of future relationship between human and machine. It is important to consider human perception of robot physical appearances,

movements, and social interactions when examining these relationships because perception can vary dramatically across cultures, generations, and genders. The goal of seamlessly integrating robots to live in harmony with humans is under exploration with robots designed to reflect human appearance, mannerisms, and motions. The resulting technological challenges include improving our understanding of human-human social interactions as well as human-machine interactions. The objective of this study was to examine neurological responses to a robot "assistant", in relation to movement, communication, and usability in a real world setting. Advanced Brian Monitoring (ABM), in collaboration with Lowe's Innovation Lab (LIL) and Neurons Inc., tested a total of N=28 subjects within San Jose, California comprising of 46.4% Female with an age range of 26-72. Participants were recruited by LIL, through a partnered external firm database whereby regular OSH shoppers, upon meeting pre-screening criteria, were asked to participate in a study. ABM's B-Alert X10 EEG headset was applied and subjects completed the ABM benchmark neurocognitive tasks: 3-Choice Vigilance Task (3CVT), Auditory Psycho-Vigilance Task (APVT), Verbal Psycho-Vigilance Task (VPVT), to individualize the model to support classification and quantification of engagement and workload. Focal attention was assessed using mobile eye-tracker during several tasks: 1) free will (find object with/without assistance-- human or robot); 2) incorporate robot's assistance; and 3)allow the subject to follow the robot with a smooth, or clunky mobile movement. Data was analyzed by events to explore neural responses for each instance of human-robot interactions. Z-scored data from the VPVT task and multiple bandwidths during the "first impression" phase revealed differences between genders; females showed higher levels of EEG-mental workload; one-way ANOVA results: $F(1, 15) = 2.69$, $p < 0.05$; Overall Slow EEG Theta: $F(1, 16) = .27$, $p < 0.05$; and Overall Slow EEG Alpha: $F(1, 16) = 1.7$, $p < 0.0$; while Males were higher in Overall EEG Delta: $F(1, 15) = 6.79$, $p < .05$; and Overall EEG Beta: $F(1, 16) = .37$. These neurophysiological and behavioral indices associated with human-robot interactions and gender can uncover aspects of social experiences to help shape the design of future robots.

Disclosures: **S.J. Smith:** None. **B.T. Stone:** None. **T. Ranatunga:** None. **A. Manna:** None. **T.Z. Ramsoy:** None. **C. Berka:** None.

Poster

546. Bipolar Disorder

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 546.01/JJJ19

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NSC 102-2410-H-431-005-MY3

Title: Motor hyperactivity and its comorbidity anxiety behavior in the animal model of ouabain-induced bipolar disorder

Authors: *Y. YING HAO[1], Y.-C. WANG[2], C. L. LAI[3], A. C. W. HUANG[1];
[1]Fo Guang University, Psychology, Yilan, Taiwan; [2]Dept. of Clin. Psychology, Fu Jen Catholic University, New Taipei City 24205, Taiwan, New Taipei City, Taiwan; [3]Program in Nutr. and Food Science, Fu Jen Catholic University, New Taipei City 24205, Taiwan, New Taipei City, Taiwan

Abstract: Previously, many studies have shown that bipolar disorder exhibited motor hyperactivity in the human and animal models. However, whether it can simultaneously exhibit the comorbidity anxiety behavior remains nuclear? The present study used various concentrations (0, 10^{-5}M, 10^{-4}M, 10^{-3}M) of ouabain, which is a Na^{+}- and K^{+}-activated adenosine triphosphatase inhibitor, to produce bipolar disorder-like symptoms in motor hyperactivity. Ouabain is often used to mimic motor dysfunction for bipolar disorder in the animal model. Also, the anxiety behavior was assessed in the present study. For the experimental procedure, all of rats were administered 5μl of artificial cerebrospinal fluid (aCSF) or 5μl of an appropriate concentration (10^{-5}M, 10^{-4}M, 10^{-3}M) ouabain solution into the left ventricle to assess motor activity and anxiety behavior. All rats received 15 min for testing motor and anxiety behaviors, simultaneously. All of rats were given 15 min for testing their motor activity and anxiety behavior in the open field task. The present results indicated that (a). mean distance travelled of the ouabain group (10^{-3}M) was longer compared with the control group (i.e., aCSF group). (b). Mean speed and maximum speed of the 10^{-3} M ouabain group was significantly increased than the other groups (including the control group). (c). Mean spent time in inside square of the 10^{-3}M and 10^{-4}M ouabain groups was significantly decreased than that of the control and 10^{-5}M ouabain groups. (d). mean inside-outside numbers of the 10^{-3}M and 10^{-4}M ouabain groups was significantly decreased than that of the control and 10^{-5}M ouabain groups. The present data indicated that the animal model of bipolar-like disorder can simultaneously show the motor function and the anxiety comorbidity symptom of bipolar disorder. The present findings are important for bipolar disorder in clinical. Keywords: ouabain, bipolar disorder, motor function, anxiety behavior NSC 102-2410-H-431-005-MY3

Disclosures: **Y. Ying Hao:** None. **Y. Wang:** None. **C.L. Lai:** None. **A.C.W. Huang:** None.

Poster

546. Bipolar Disorder

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 546.02/JJJ20

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: The change in expression of hippocampal cholinergic neurostimulating peptide precursor affects psychiatric behavior

Authors: *M. MIZUNO[1], T. SATO[2], D. KATO[2], T. TOYODA[2], N. MATSUKAWA[2]; [1]Nagoya City Univ., Nagoya-Shi, Japan; [2]Neurol. and Neurosci., Nagoya City Univ., Nagoya-shi, Japan

Abstract: Hippocampal cholinergic neurostimulating peptide (HCNP) induces the synthesis of acetylcholine in the medial septal nucleus in vitro and in vivo. HCNP has precursor protein, HCNPpp, which was also reported as a multifunctional protein, participating in signal pathways and influencing in the function of G-protein-coupled receptors. We recently demonstrated that HCNPpp transgenic mouse had a psycho-behavior character of a depressive-like state in tail suspension test (TST). In this study, to further examine whether HCNPpp may be involved in neuropsychiatric behavior or not, we investigated tail suspension test (TST) in HCNPpp conditional knockout (KO) mice by using Cre recombinase transgenic mice driven by Calmodulin kinase II (CaMKII) promoter. Further insight of the period influence of HCNPpp reduction, we also examined TST in mice with HCNPpp knockdown in the hippocampus at 12-14 week-old by adeno-associated virus (AAV). Here, we demonstrated that decrease of HCNPpp in the hippocampus at 12-14 week-old may be involved in the disease development of mania-like behavior. We confirmed the decrease of gamma-aminobutyric acid (GABA) A receptor alpha 3 subunit in this model mice. These findings suggested that HCNPpp in the hippocampus may be involved in bipolar disorder, depressive-like and/or mania-like behavior.

Disclosures: **M. Mizuno:** None. **T. Sato:** None. **D. Kato:** None. **T. Toyoda:** None. **N. Matsukawa:** None.

Poster

546. Bipolar Disorder

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Program#/Poster#: 546.03/JJJ21

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: T32 DA031111

R01 DA039865

Title: Altered excitatory synaptic activity of nucleus accumbens neurons in a genetic mouse model of mania

Authors: *P. K. PAREKH, D. BECKER-KRAIL, Y. HUANG, C. MCCLUNG;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: It is well established that the circadian molecular clock regulates monoaminergic systems controlling mood, anxiety and motivated behaviors. Disruptions in the circadian gene, *Clock*, are associated with increased risk for bipolar disorder (BD) in humans and manic-like behaviors as well as hyperdopaminergia have been characterized in *Clock*α19 mutant mice. While gross neural circuit function within mesocorticolimbic regions, particularly the nucleus accumbens (NAc), appears to be disrupted in this model, the underlying molecular and synaptic mechanisms have not been studied. Here we show that *Clock*α19 mice display reduced AMPA-receptor mediated excitatory synaptic responses (mEPSCs and EPSCs) at NAc medium spiny neurons (MSNs) across the light/dark cycle compared with wildtype (WT) littermates. We find that these alterations are likely postsynaptic as presynaptic release of glutamate onto MSNs is not disrupted in *Clock* mice. Additionally, we demonstrate that NAc surface levels of the AMPA receptor subunit, GluA1, are decreased in mutant mice compared with WT at both light and dark phases, consistent with reduced functional synaptic response. We also explored diurnal differences in the intrinsic membrane excitability of NAc MSNs in mutant and WT mice and found no change in firing rate, however we observed a significantly hyperpolarized resting membrane potential of *Clock*α19 MSNs suggesting lowered excitability. Lastly, we investigated whether overexpression of synaptic proteins, GluA1 and PSD-95, in the NAc of mutants could normalize manic-like behavior. These findings demonstrate that a deficient *Clock* gene results in alterations in NAc glutamatergic transmission and synaptic strength potentially as a consequence of increased dopaminergic tone.

Disclosures: P.K. Parekh: None. **D. Becker-Krail:** None. **Y. Huang:** None. **C. McClung:** None.

Poster

546. Bipolar Disorder

Location: Halls B-H

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Program#/Poster#: 546.04/JJJ22

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: JSPS KAKENHI Grant Number JP15K19753

Title: Neurodevelopmental differences underlie discordant psychosis in a pair of monozygotic twins

Authors: *T. SAWADA[1], T. E. CHATER[2], Y. GODA[2], T. KATO[1];
[1]Lab. for Mol. Dynamics of Mental Disorders, [2]Lab. for Synaptic Plasticity and Connectivity, RIKEN Brain Sci. Inst., Wako, Saitama, Japan

Abstract: Schizophrenia (SZ) and bipolar disorder (BP) are neuropsychiatric diseases characterized by psychotic and/or mood symptoms. Despite extensive studies, the cellular basis of these disorders is still not elucidated, partly because of the difficulty in analyzing patients' neurons at the cellular level. Though induced pluripotent stem cell (iPSC) technology is expected to provide a clue for understanding the neurobiological basis of psychoses, determination of subtle disease-associated cellular phenotypes is complicated by the large variability between individuals. Here, we generated iPSC-derived neural cells from a pair of monozygotic twins discordant for bipolar-type schizoaffective disorder that shares the clinical features with both SZ and BP, to understand the cellular basis of psychosis. Multiple iPSC clones were differentiated into neural cells by using the SFEBq (serum-free floating culture of embryoid body-like aggregates, quick) method in combination with dual-SMAD inhibition. iPSCs of both twins differentiated into neural stem/progenitor cells (NSPCs) expressing specific cell surface markers such as SSEA-1 and PSA-NCAM. However, the affected twin's aggregates were slightly but significantly smaller than the unaffected twin. Moreover, Nestin-positive NSPCs of the affected twin migrated more than the unaffected twin's cells. RNA sequencing-based transcriptomic analysis of neural cells revealed the increased expression of genes involved in cortical development such as *TBR1*, *DCX* and *RELN* in the affected twin-derived cells. Additionally, expression of genes critical during early developmental stages for neural patterning and specification were also different between the twins. Consistent with the previous studies, several genes involved in ventralization of the telencephalon were upregulated in the affected twin-derived neural cells that were also accompanied by increased expression of GABAergic neuron markers, such as *GAD1/2*.
These findings suggest that early neurodevelopmental differences underlie discordant psychosis between the twins. We are presently determining the effect of these changes on the function and morphology of neurons after culturing for an extended period (up to 120 days) and searching for fundamental causes of the discordance, including epigenetic differences.

Disclosures: T. Sawada: None. **T.E. Chater:** None. **Y. Goda:** None. **T. Kato:** None.

Poster

546. Bipolar Disorder

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant MH77681

NIH Grant MH105824

NIH Grant DA033945

Title: Cholinergic innervation of the hippocampus regulates behaviors related to depression and resilience to social stress.

Authors: *Y. S. MINEUR, T. MOSE, D. THOMPSON, C. PINEDA, M. BENTHAM, M. R. PICCIOTTO;
Psychiatry, Yale Univ. Sch. Med., New Haven, CT

Abstract: Cholinergic signaling is critical for regulation of normal mood and associated psychiatric disorders. Clinical imaging studies suggest that nicotinic acetylcholine receptor (nAChR) occupancy by acetylcholine (ACh) is increased in the brains of human subjects with depression or bipolar disorder during a depressive episode. Conversely, blockade of either nicotinic or muscarinic ACh receptors can have antidepressant effects in human subjects and in preclinical models. Stress can increase ACh levels in specific brain areas and alter acetylcholinesterase (AChE) activity. Further, increasing ACh signaling systemically or locally in the hippocampus can induce behaviors related to anxiety and depression. These studies suggest that cholinergic innervation of the hippocampus is involved in behaviors related to anxiety and depression, but the role of specific cholinergic inputs to the hippocampus in mediating depression-like behaviors remains unknown.
With the idea that environmental stimuli that can induce depression might increase ACh signaling in hippocampus by decreasing its breakdown, we first measured AChE activity in prefrontal cortex and hippocampus in C57BL/6J mice following restraint stress. Stress had significant region- and sex-dependent effects on AChE activity, with the greatest regulation occurring in ventral hippocampus. We next silenced or activated the cholinergic neurons innervating the hippocampus by infusing AAV-floxed-Gi-DREADD or AAV-floxed-Gq-DREADDs locally into the medial septum or hippocampus of Chat-CRE mice followed by peripheral administration of CNO to modulate the activity of cholinergic inputs or intrinsic hippocampal cholinergic interneurons, respectively. We then evaluated the effects on depression-like behaviors and response to social stress. While there was little effect of stimulating or inhibiting the medial septum, which provides about 70% of the cholinergic input to the hippocampus, stimulating the sparse population of ChAT-Cre-positive cells in the hippocampusin mice with AAV-floxed-Gq-DREADD using CNO increased anxiety-like behaviors, immobility in tests of antidepressant efficacy and interaction in the social defeat paradigm.
These results demonstrate that stress can alter expression of biochemical pathways that influence cholinergic signaling in the hippocampus. Further, the current study suggests that activating the ChAT-positive neurons in the hippocampus, but not the inputs from the medial septum/diagonal bands of Broca, can induce behaviors related to anxiety and depression in the mouse.

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Poster

546. Bipolar Disorder

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: CNPq Grant 308412/2015-0

Title: Quercetin reduces manic-like behavior and brain oxidative stress in animal models of mania

Authors: *R. ANDREATINI[1], K. K. S. KANAZAWA[2], D. D. VECCHIA[2], E. M. WENDLER[2], P. A. S. HOCAYEN[2], F. A. R. LÍVERO[2], M. C. STIPP[2], I. M. R. BARCARO[2], A. ACCO[2]; [2]Pharmacol., [1]Univ. Federal Do Paraná, Curitiba, Brazil

Abstract: Despite the availability of many pharmacological agents for treating mania in bipolar disorder (BD), their management remains a challenge for various reasons (e.g. refractoriness side effects or relapse). Therefore, it is important to search new drugs with antimanic properties. Increase in protein kinase C and oxidative stress have been related to mania, so drugs with antioxidant or PKC inhibitory activity may have antimanic effect. The flavonoid quercetin has antioxidant and PKC-inhibiting activities, which resemble lithium, the first-line treatment for mania in BD. Thus, we hypothesized that quercetin may has antimanic-like effects.
In the present study, manic-like behavior (hyperlocomotion) and oxidative stress were induced in male Swiss mice by 24 h paradoxical sleep deprivation (PSD) or methylphenidate (5 mg/kg, i.p.) administration. Brain oxidative stress was measured by reduced glutathione (GSH) and lipid peroxidation (LPO) levels in the prefrontal cortex, hippocampus and striatum.
Lithium (100 mg/kg i.p.) and quercetin (10 and 40 mg/kg) prevented PSD-induced hyperlocomotion. Quercetin also reversed: PSD-induced decrease in GSH levels in the prefrontal cortex and striatum; PSD-induced increase in LPO in the prefrontal cortex, hippocampus, and striatum. In PSD mice Pearson's correlation analysis revealed a negative correlation between locomotor activity and GSH in the prefrontal cortex and a positive correlation between locomotor activity and LPO in the prefrontal cortex and striatum. Acute and chronic treatment with lithium reduced methylphenidate-induced hypercomomotion. Chronic, but not acute, treatment with quercetin (10 and 40 mg/kg) blocked methylphenidate-induced hyperlocomotion. In addition, chronic treatment with lithium or quercetin blocked methylphenidate-induced increase in LPO levels in the striatum. There was a positive correlation between

hyperlocomotion and LPO levels in the prefrontal cortex. Also, there was a negative correlation between hyperlocomotion and GSH levels in the striatum. All behavioral effects of lithium and quercetin were seen at dose that did not change spontaneous locomotor activity.
These results suggest an antimanic-like effect of quercetin and the antioxidant action of quercetin might contribute to its antimanic-like effects. It is interesting to note that quercetin has already studied in clinical trials (e.g. for sarcoidosis) and that the dose use in the present study is below the dose range tested in these clinical trials.

Disclosures: R. Andreatini: None. **K.K.S. Kanazawa:** None. **D.D. Vecchia:** None. **E.M. Wendler:** None. **P.A.S. Hocayen:** None. **F.A.R. Lívero:** None. **M.C. Stipp:** None. **I.M.R. Barcaro:** None. **A. Acco:** None.

Poster

546. Bipolar Disorder

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH BD2K U54EB020403

NIH R01EB015611

NIH R01MH085667

Title: Cortical abnormalities in patients with bipolar disorder more localized than in those with schizophrenia

Authors: *J. FASKOWITZ[1], F. PIZZAGALLI[2], B. MWANGI[3], P. KOCHUNOV[4], P. M. THOMPSON[2], J. C. SOARES[3], N. JAHANSHAD[2];
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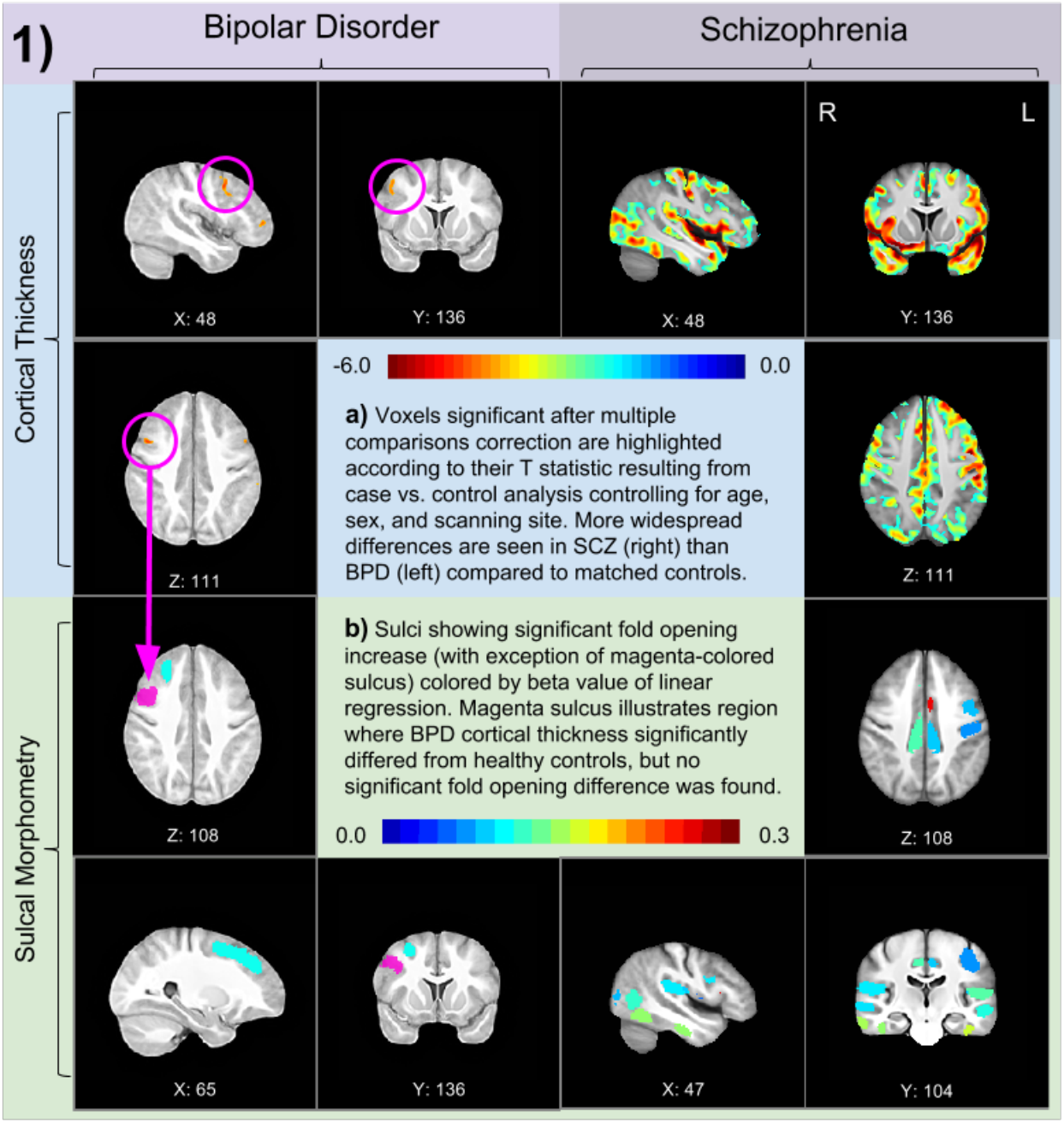
Abstract: Background: Bipolar disorder (BPD) and schizophrenia (SCZ) share partially overlapping neuropsychiatric symptom profiles, and the disorders also have overlapping genetic and environmental risk factors. Gray matter abnormalities are implicated in both disorders, but the nature and extent of overlap is still poorly understood. Here, we create a framework to map detailed patterns of cortical differences in multi-site MRI studies of each patient group compared to matched controls. We map out cortical thickness differences on a voxelwise level, and

examine detailed morphometric properties of the sulcal patterns, specifically the degree of fold opening. Methods: We analyzed cortical thickness and sulcal morphometry from T1-weighted brain MRI scans of patients diagnosed with BPD-I compared to healthy controls (HC), across 4 scanners (N: 183; cases: 108; mean age: 35.8 +/- 11.4; 60.1% F); we performed the same analysis comparing SCZ patients to HC, with data collected across 3 scanners (N: 386; cases: 386; mean age: 40.5 +/- 12.8; 41.7% F). Cortical thickness was computed using an ANTs pipeline with site-specific templates; sulcal morphometry was conducted with ENIGMA protocols built from FreeSurfer and BrainVISA tools. When comparing patients to controls, we adjusted for age, sex, and scanner. 10,000 permutations were run on the sulcal comparisons to obtain non-parametric estimates of significance for fold opening in 68 sulci; we corrected for multiple comparisons with FDR ($q<0.05$). Results: We observed a significantly greater fold opening of the right superior frontal sulcus and thinner cortex in the right intermediate pre-central gyrus in patients with BPD compared to HC (Figure 1, left). In SCZ patients however, greater fold opening was observed for 33 sulci and thinner gray matter was observed globally, with the greatest effects in the insula (Figure 1, right). Conclusion: Further work on the differences in neuro-circuitry between these groups of patients and controls may shed light on the overlapping symptoms and neurocognitive outcomes for patients with these disorders.



Disclosures: **J. Faskowitz:** None. **F. Pizzagalli:** None. **B. Mwangi:** None. **P. Kochunov:** None. **P.M. Thompson:** None. **J.C. Soares:** None. **N. Jahanshad:** None.

Poster

546. Bipolar Disorder

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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Title: How does sexual dimorphism in the brain's connectivity and structure relate to mood disorder symptoms in children?

Authors: *O. R. PHILLIPS[1], A. ONOPA, K[2], V. HSU[3], J. HALLMAYER[4], I. GOTLIB[5], L. MACKEY[6], M. K. SINGH[7];
[1]Neurology, neuroscience, [3]Mathematical and Computat. Sci., [4]Dept. of Psychiatry, Div. of Child and Adolescent Psychiatry, [5]Dept. of Psychology, [6]Statistics, [7]Dept. of Psychiatry, Div. of Child and Adolescent Psychiatry, Stanford Univ. Sch. of, [2]Stanford Univ., Stanford, CA

Abstract: Introduction:
Sex differences in psychiatric disorders are common and can emerge in childhood. Girls typically experience internalizing disorders (e.g. depression, anxiety), whereas boys experience externalizing disorders (e.g., ADHD, ODD). However, the human brain is not strictly male or female. In order to gain a more nuanced understanding of the role of sex in psychopathology, we investigated features that best discriminated male and female brains and their relationship to internalizing and externalizing psychopathology.
Methods:
Using data from the Philadelphia Neurodevelopmental Cohort (8719, 13.76±3.68, 4498F/4221M), we performed a factor analysis on the 256 psychopathological variables using regression. Two of the factors that emerged corresponded to internalizing and externalizing symptoms. Structural MRI MPRAGE data (977, age 14.03±3.50, 512F/465M) was run through Brainsuite and registered to the Colin-brain atlas. Cortical thickness values for sixty-four regions of interest were then extracted for each subject. We estimated the sexual dimorphism scores of each subject's cortical thickness regions of interest using the likelihood ratio of the male and female volume distributions. All sixty-four regions were then ranked according to their level of sexual dimorphism. Finally, we then performed a partial correlation analysis with age as a covariate between the factor scores and the top five most sexually dimorphic regions.
Results:
Correlations between the likelihood ratio of sexual dimorphism for the top five most dimorphic

regions and internalizing and externalizing factor scores are shown in Table 1:
Table 1: Correlations Between Internalizing and Externalizing and sexual dimorphism scores

Region:	Internalizing Score		Externalizing Score	
	r	p	r	p
Right Angular gyrus	0.123	**0.001**	-0.123	**0.001**
Right Supramarginal gyrus	0.110	**0.002**	-0.114	**0.001**
Right Lingual gyrus	0.069	0.054	-0.071	**0.048**
Right Fusiforme gyrus	0.102	**0.004**	-0.102	**0.004**
Right Pre-cuneus	0.38	0.283	-0.40	0.268

Discussion:
The following findings emerged from this study: (1) increased "femaleness" of the cortical gray matter thickness in the most dimorphic regions was correlated with increased "internalizing" symptoms (e.g. depression, anxiety), (2) increased "maleness" of the cortical gray matter thickness in the most dimorphic regions was correlated with "externalizing" symptoms (e.g., ADHD, ODD). Thus, a non-dimorphic quantification of the brain's sex can yield insights into internalizing and externalizing symptoms.

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Poster

546. Bipolar Disorder

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Program#/Poster#: 546.09/JJJ27

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH HHSN271201300030C

Title: A neuropathological study of the comorbidity of bipolar disorder and Alzheimer's disease

Authors: *K. M. SULLIVAN[1], J. GONZALEZ[1], H. PANTAZOPOULOS[2,5], D. HARPER[3,5], B. FORESTER[3,5,7], T. WOO[1,4,5], S. BERRETTA[1,2,5,6];
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Abstract: Introduction: Emerging evidence suggests that individuals with bipolar disorder (BD) have an increased risk of developing Alzheimer's disease (AD) and non-specific dementia compared to the normal population. However, it is not yet known whether these clinical observations underlie increased severity of neuropathological changes typical of AD. Notably, chronic lithium exposure may decrease this risk, potentially exerting a neuroprotective effect in both BD and AD patients. The present postmortem studies test the hypothesis that BD subjects may present with earlier onset and/or increased severity of AD pathology with respect to control subjects. lithium exposure in BD subjects is expected to be inversely correlated to the severity of neuropathology. **Methods:** BD subjects (n=78), AD subjects (n=72) and healthy controls (n=81) were matched by age and gender. Neuropathological data such as Braak and Braak stage, presence of infarcts, small vessel disease, amyloid angiopathy, and athero/arteriosclerosis were collected from neuropathology reports. Exposure to lithium, lifetime and within 6 months prior to death, was collected for BD subjects (n=55). Potential confounds such as age, gender, brain weight, and cause of death were tested using stepwise regression analysis. **Results:** As expected, Braak and Braak stages were significantly higher in subjects with AD as compared to those with BD or controls (p=<.0001). Comparisons between healthy control and BD subjects did not show significant differences with regard to Braak and Braak stages. This result did not change when including lifetime lithium exposure as a covariate. Frequency of amyloid angiopathy was significantly higher in AD subjects (p=<.0001) with respect to the other two groups. There is no significant difference between AD, BD, and control subjects in prevalence of athero/arteriosclerosis, infarct, and small vessel disease. A positive correlation observed between lifetime lithium exposure and Braak and Braak severity (p=0.0318). Further testing shows that such positive correlation is an effect of age which is positively correlated with both Braak and Braak scores as well as lifetime lithium exposure. Presence of amyloid angiopathy, athero/arteriosclerosis, infarct, and small vessel disease in relation to lifetime exposure to lithium was not significant. **Conclusions:** It was found that there was no difference between BD subjects and controls in any of the dementia-related pathologies investigated. These results suggest that chronic exposure to lithium in subjects with BD may not significantly protect from these neuropathological changes commonly observed in subjects with dementia.

Disclosures: K.M. Sullivan: None. **J. Gonzalez:** None. **H. Pantazopoulos:** None. **D. Harper:** None. **B. Forester:** None. **T. Woo:** None. **S. Berretta:** None.

Poster

546. Bipolar Disorder

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 546.10/JJJ28

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: BJD School of Pharmacy Professional Practice Plan 2016

Title: Evaluation of BDNF and GSK3beta gene polymorphism frequencies in bipolar and control groups.

Authors: *D. MEROLA, R. S. KIDD, A. ZIVANOVIC, M. LIZER, N. HENGEN;
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Abstract: Background: Linking genetic variations and psychiatric illnesses is a crucial first step towards elucidating the pathogenesis and varied treatment response of mental disorders. Emerging evidence has revealed that neurotrophic signaling pathways may be implicated in the pathophysiology of bipolar disorder. Two important components of this signal transduction cascade, Brain-derived neurotrophic factor (BDNF) and Glycogen synthase kinase-3beta (GSK-3β), are linked through crosstalk. In this study, we evaluate the relationship of BDNF and GSK-3β polymorphisms in bipolar disorder and control subjects.
Objective: The purpose of this study was to evaluate and compare the genotype frequencies, allele frequencies, and association of five single nucleotide polymorphisms (SNPs) of the GSK-3β gene and one SNP of the BDNF gene in bipolar disorder and control populations.
Methods: The genotype and allele frequencies for SNPs rs6265, rs6438552, rs334558, rs12630592, rs3755557, and rs2199503 were evaluated in bipolar disorder (n=101) and control (n=103) subjects using real-time PCR. Pearson's Chi-square test was used to compare genotype and allele frequencies in each population. Logistic regression was performed to assess the association of all of these SNPs with bipolar disorder.
Results: A statistically significant difference in allele frequency was observed for GSK-3β SNP rs2199503T>C between bipolar and control groups. The frequency of the C allele was 0.257 in the bipolar group compared to 0.364 in the control group (p=0.02). Significant differences were also observed in allele frequencies for BDNF SNP rs6265C>T. The frequency of the T allele was 0.149 in the bipolar group compared to 0.228 in the control group (p=0.04). The strongest associations in the logistic regression were also identified with rs2199503 (p=0.033) and rs6265 (p=0.042). These two SNPs were found to be significantly associated with bipolar disorder; rs2199503 yielded in an OR = 1.543 (95% CI of 1.036-2.298) and rs6265 had an OR = 1.697 (95% CI of 1.018-2.827).
Conclusions: Rs2199503 is a GSK-3β gene intron 1 region variant that has been associated with schizophrenia and major depressive disorder in one prior study. Rs6265 is a BDNF non-

synonymous SNP that results in a Val>Met substitution. The Met allele has been associated with decreased BDNF activity. Association of these SNPs with our bipolar disorder group warrants further investigation to determine the neurobiological effects of these SNPs and elucidate potential clinical implications.

Disclosures: D. Merola: None. **R.S. Kidd:** None. **A. Zivanovic:** None. **M. Lizer:** None. **N. Hengen:** None.

Poster

546. Bipolar Disorder

Location: Halls B-H

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Program#/Poster#: 546.11/JJJ29

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: RO-1 MH-080193-05

RO-1 MH-059852

Title: GSK-3β signaling pathway is altered in olfactory neuro-epithelial cells of bipolar patients

Authors: *R. RAY, T. MCCLOSKEY, M. BARRETT, N. MIRZA, M. THASE, K. BORGMANN –WINTER, C.-G. HAHN;
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Abstract: Glycogen synthase kinase -3(GSK-3), encoded by two separate genes GSK-3α and GSK3β, is a constitutively active, serine /threonine kinase, which is highly abundant in brain. GSK-3 activity is modulated by phosphorylation of the S^9 and Y^{216} residues; the former decreases and the latter increases the enzyme activity. GSK-3 modulates a variety of neuronal functions including neurotransmission, neurite outgrowth and synaptic plasticity. Multiple lines of evidence suggest altered GSK-3 function as a pathophysiologic mechanism of bipolar disorder. Nevertheless, molecular underpinnings for such dysregulation and their functional consequences in neural cells of patients are presently unknown. Previous studies have focused on phosphorylation of the S^9 residue and showed a decrease in BD cases, which predicted increased GSK-3β activity. The goal of this study was to further delineate GSK-3 dysregulation in BD by examining phosphorylation of both residues as well as GSK-3 activity in an isoform specific manner, in neural cells of patients. We examined olfactory neuro-epithelial cells (NE), the only neural cells derived from patients without genomic reprogramming for the current study. In olfactory NE cells , derived from 13 age- and sex- matched pairs, we found a significant increase of pY^{216}GSK3β ($p=0.04$, $t=2.2$, $df=12$) in NE cells of BD cases, while p-S^9GSK-3β level was

comparable. Consistent with this result, GSK-3β activity was significantly increased (p=0.02, t=2.66, df=11) in NE cells from BD patients. We then examined the effects of chronic lithium treatment (3mM for 5 days) in olfactory NE cells. As shown in previous studies, lithium treatment increased the level of p-S^{9}GSK-3b in both healthy and BD subjects (p=0.03, t=2.4, df=12 and p=0.01, t=2.26, df=12 respectively). We observed differential responses to the agent between the BD and healthy subjects; the level of p-Y^{216}GSK3β and the activity of GSK-3β were increased significantly(p=0.03,t=2.39, df=12 and p=0.0027, t=3.84,df=11 respectively) by lithium treatment only in healthy subjects but not in BD patients. Together, these results offer direct evidence for increased GSK-3β activity in NE cells of patients accompanied by parallel increases in pY^{216}GSK3β prior to lithium treatment and differential regulation of p-Y^{216}GSK3β in response to lithium. To assess functional consequences of these dysregulations, the phosphorylation status of the substrates for GSK-3b is presently investigated.

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Poster

547. Pain, Headache, Migraine, and Trauma

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 547.01/JJJ30

Topic: G.06. Post-traumatic Stress Disorder

Support: ARO-PTSD grant

Title: Molecular indicators of stress-induced inflammation in patients with post-traumatic stress disorder and in rodent models

Authors: *S. MUHIE[1], A. GAUTAM[2], N. CHAKRABORTY[1], R. HAMMAMIEH[2], J. MEYERHOFF[1], M. JETT[2];
[1]Systems Biol. Program/Genevausa, Fort Detrick, MD; [2]Systems Biol. Program, USACEHR, Fort Detrick, MD

Abstract: Gene expression, DNA methylation and microRNA (miR) datasets from patients with post-traumatic stress disorder (PTSD) and from rodent (mouse and rat) models of PTSD were collected from the Gene Expression Omnibus. After quality assessment and normalization, we used two datasets of DNA methylation, three gene expression datasets and one miR dataset from human patients, and four datasets from brain regions of rat and mouse models for downstream analysis. Differentially expressed transcripts and methylated promoter regions across the different datasets were found to be commonly associated with activated inflammatory responses.

Other pathways associated with differentially changed molecules include mTOR signaling, oxidative stress, WNT signaling, insulin signaling, type II diabetes, transcription, chromatin modification, neurogenesis and synaptic plasticity. Our aggregate and meta-analyses of these datasets suggest that stress-induced inflammation may be negatively affecting brain regions responsible for cognition and traumatic fear memory extinction, leading to responses which modulate behavioral and systemic disorders (such as metabolic disorders, immune dysfunction and cardiovascular problems). Though it is difficult to infer the causal order of PTSD-associated pathologies, at the transcriptome and epigenome levels, inflammation seems to trigger cascades of PTSD-related disorders. A feedback loop is also highly probable between the different disorders and inflammatory responses whereby comorbid conditions can aggravate anxiety disorders.
Disclaimer: The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation.

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Poster

547. Pain, Headache, Migraine, and Trauma

Location: Halls B-H

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Program#/Poster#: 547.02/JJJ31

Topic: G.06. Post-traumatic Stress Disorder

Support: Ontario Mental Health Foundation

Ryerson University

Canadian Institutes of Health Research

National Institute of Mental Health

Title: Posttraumatic stress disorder-related brain network organization: a novel combined approach using graph theory and scaled subprofile modeling

Authors: *J. KO[1], T. A. GIRARD[3], C. MONSON[3], R. PATEL[2];
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Abstract: INTRODUCTION
Posttraumatic stress disorder (PTSD) is prevalent mental illness (~9% life-time) that is caused by exposure to trauma involving death or the threat of death, serious injury, or sexual violence. As it is associated with vastly heterogeneous origins, accurate diagnosis and optimal treatment strategies are sometimes very difficult to achieve. As in many other mental disorders, no known biomarker exists, which makes it difficult to assess treatment response and to determine when to return to work. Recent brain imaging studies elucidated the underlying neural underpinnings of PTSD, mainly involving hyper-active limbic brain regions and mal-functioning top-down cognitive control of prefrontal cortices (Patel et al., Neurosci Biobehav Rev, 36:2130-2142, 2012). Functional connectivity analysis using graph theory has shed lights on how alterations of brain network organization affect brain function and ultimately translated to symptom expressions in brain disorders (Rubinov and Sporns, Neuroimage, 52:1059-1069, 2010).
METHOD
Here we proposed a novel approach combining graph theory' eigenvector centrality and scaled subprofile modeling (Spetsieris et al., J Vis Exp, 2013) to identify a disease-related covarying pattern of information flow in the context of brain network organization. Twenty two patients (11 PTSD and 11 controls) were analyzed using the proposed analysis pipeline.
RESULT
We have identified a spatial pattern of eigenvector centrality which was significantly increased in PTSD vs. control ($p=0.043$). The pattern was characterised by increased eigenvector centrality in the orbitofrontal regions, amygdala, anterior cingulate, middle frontal and angular cortices, and decreased eigenvector centrality in the paracentral lobule, inferior occipital, pre- and post-central, Rolandic operculum, fusiform gyrus and cerebellar lobule 4/5. Interestingly, this pattern expression was correlated with better memory performance on negative valence items vs. positive valence items only in PTSD ($p=0.034$) but not in controls ($p=0.664$).
DISCUSSION
While further studies are warranted, the proposed analytic method may produce an objective neuroimaging-based biomarker, which may benefit clinicians, patients and caregivers by significantly advancing our ability to establish a connection between brain-related changes and an improvement in clinical symptoms.

Disclosures: J. Ko: None. **T.A. Girard:** None. **C. Monson:** None. **R. Patel:** None.

Poster

547. Pain, Headache, Migraine, and Trauma

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 547.03/JJJ32

Topic: G.06. Post-traumatic Stress Disorder

Support: The William Anderson Chair in PTSD Research, University of Minnesota

Title: Cognitive function in veterans with posttraumatic stress disorder (PTSD)

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Abstract: The Montreal Cognitive Assessment (MoCA) is a brief screening instrument that is widely used in clinical and research settings to evaluate for mild cognitive dysfunction. Diverse conditions including Parkinson's disease, Alzheimer's disease, traumatic brain injury, depression, schizophrenia, and substance use disorders have been associated with impaired performance on the MoCA. To date, no published studies have evaluated MoCA performance in individuals with posttraumatic stress disorder (PTSD). In the present study, 175 veterans with PTSD and 231 healthy control veterans completed the MoCA as part of a larger study on brain functioning and PTSD. Results indicated that both groups performed in the "normal" range; nevertheless, the MoCA total score, after controlling for group differences in age, was significantly lower in veterans with PTSD relative to healthy control veterans (p = .027). Multivariate analyses demonstrated that impairments on the abstraction (p = .017) and delayed recall (p= .034) domains accounted for the lower total score in veterans with PTSD. Notably, group differences were not accounted for by depression. Although veterans with PTSD may not exhibit broad cognitive dysfunction, relative deficits may impart specific functional impairment.

Disclosures: R. Johnson: None. **L.M. James:** None. **B. Engdahl:** None.

Poster

547. Pain, Headache, Migraine, and Trauma

Location: Halls B-H

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Program#/Poster#: 547.04/JJJ33

Topic: G.06. Post-traumatic Stress Disorder

Title: Regionally specific cortical alpha-band asymmetry in mild traumatic brain injury with significant posttraumatic stress disorder symptoms: a magnetoencephalographic study

Authors: *J. D. HUGHES[1,2], M. POPESCU[2], A.-E. POPESCU[2], T. J. DEGRABA[2];
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Abstract: Hemispheric asymmetries of EEG recorded alpha-band power in prefrontal cortices are indicative of trait differences in emotional response biases, based on differences in

hemispheric specialization in the processing of emotional valence and motivational direction. Studies have consistently demonstrated relatively greater alpha-band power recorded over left versus right frontal scalp electrodes in the setting of depression and anxiety. Despite the overlap in symptoms of these disorders with posttraumatic stress disorder (PTSD), no consistent hemispheric alpha asymmetry has been demonstrated in PTSD. However, in the case of EEG recordings: 1) prefrontal region specific asymmetries in opposite directions could result in cancellation of effects due to spatial averaging and 2) an asymmetry limited to orbitofrontal or ventromedial prefrontal cortices would likely be undetected due to the significant distance from scalp electrodes. Magnetoencephalography with source reconstruction methods would be better suited to detect asymmetries in these circumstances. Therefore, we sought to localize region specific differences in alpha-band power asymmetry between groups of mTBI patients with or without significant PTSD symptoms utilizing MEG, with particular interest in orbitofrontal and ventromedial prefrontal cortices. All 30 participants had a history of mTBI. PCLM-scores were used to assign patients to two groups based on the severity of their PTSD symptoms (high versus low). Resting-state MEG recordings (5 min duration, eyes closed) were filtered in alpha band (8 to 13 Hz). The power of the estimated current sources was integrated over 68 cortical regions. Region wise inter-hemispheric asymmetry coefficients were compared between groups using non-parametric Wilcoxon rank-sum tests. Patients with high PTSD symptom severity demonstrated a relative increase in mean alpha power of left versus right hemisphere over medial brain areas including the posterior cingulate, paracentral and superior frontal regions, similar to previously reported findings using EEG recordings in patients with depression and anxiety. Additionally, we found a relative decrease in mean alpha power of left versus right hemisphere in orbitofrontal cortex and inferior frontal gyrus, regions directly involved in the modulation of emotional responses to stimuli, in patients with severe PTSD symptoms. To our knowledge, this is the first study to demonstrate an alpha-band asymmetry specific to orbitofrontal cortex in any patient population. The findings in this study may provide novel insights into the pathophysiology of PTSD in patients with a history of mTBI.

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Poster

547. Pain, Headache, Migraine, and Trauma

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Topic: G.06. Post-traumatic Stress Disorder

Support: National Council for Sci-entific and Technological Development (CNPq)

Carlos ChagasFilho Foundation of Research Support in Rio de Janeiro (FAPERJ)

Coordination for the Improvement of Higher Education Person-nel (CAPES)

Title: Brain structural alterations associated to peritraumatic tonic immobility in victims of urban violence with posttraumatic stress disorder.

Authors: V. ROCHA-REGO, C. M. FRANKLIN, I. FIGUEIRA, *E. VOLCHAN;
Federal Univ. of Rio De Janeiro, Rio De Janeiro, Brazil

Abstract: Tonic immobility, the last-ditch defense under mortal threat, is characterized by profound motor inhibition and is known to increase the chances of surviving an attack. Tonic immobility may be elicited by intense and inescapable life threats. Psychometric measures in patients with posttraumatic stress disorder revealed the occurrence of peritraumatic tonic immobility associated with the severity of symptoms and refractoriness to pharmacological treatment. Postural and cardiac recordings from patients in response to autobiographical trauma audio-play revealed reduction of body sway and intense tachycardia associated with reports of tonic immobility, meaning that tonic immobility reaction can be induced in a laboratory setting, an otherwise safer environment. Here we conducted a voxel based-morphometry study (VBM/DARTEL) to investigate brain structural alterations associated with peritraumatic tonic immobility scores in a sample of victims of urban violence (mostly armed robbery) with posttraumatic stress disorder (N=16). By regressing gray mater volume against overall peritraumatic tonic immobility scores, we found a significant negative correlation in left extrastriate body area, right paracentral/precuneus, right frontal superior and right inferior temporal gyrus. These areas have been implicated in the processing of social cues and could be relevant to the interpretation of interpersonal threats. The present results suggest that PTSD patients with higher reports of peritraumatic tonic immobility present less gray matter volume in those areas. Brain abnormalities in those areas could lead to errors in the distinction of an actual interpersonal violence from a non risky trauma-related cue, leading to recurrent episodes of tonic immobility.

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Poster

547. Pain, Headache, Migraine, and Trauma

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Program#/Poster#: 547.06/JJJ35

Topic: G.06. Post-traumatic Stress Disorder

Support: Robert M. McCormick Tribune Foundation

Julius N. Frankel Foundation

Joseph G. Nicholas Foundation

Title: Examining the relationship between PTSD and cognition among military veterans: Does gender make a difference?

Authors: *K. MONTRY[1], C. STARR[2], E. LARSON[3];
[1]Psychology, Rosalind Franklin Univ. of Med. & Sci., Chicago, IL; [2]Univ. of California Santa Cruz, Santa Cruz, CA; [3]Rehabil. Inst. of Chicago, Chicago, IL

Abstract: Posttraumatic stress disorder (PTSD) is associated with neurocognitive dysfunction across multiple cognitive domains, including memory, processing speed, attention, and various executive functions. Most studies of neurocognitive outcomes in PTSD have focused on mostly male samples and it remains unclear whether sex differences exist within these outcomes. The present study examined PTSD diagnosis, sex, and performance across five neuropsychological domains of the Repeatable Battery for Assessment of Neuropsychological Status (the RBANS) in a sample of 228 veterans. Bivariate analysis showed both sex and PTSD diagnosis each had significant relationships with performance on RBANS Visuospatial/Visuoconstructional Index. To further examine the relative contribution of sex and PTSD diagnosis to visuospatial abilities, we conducted two hierarchical regression analyses. The first analysis showed that after controlling for individual subject characteristics (i.e., premorbid intellectual ability, educational attainment, effort, age) and for PTSD diagnosis, sex continued to significantly contribute unique variance in visuospatial/visuoconstructional abilities, with females performing lower in this cognitive domain than males. Similarly, a second analysis of the same sample showed that after controlling for individual subject characteristics and sex, PTSD diagnosis significant contributed unique variance in visuospatial / visuoconstructional abilities. A comparison of the two predictive models shows that sex was a stronger predictor of visuospatial abilities than PTSD diagnosis. The present findings suggest that the neurocognitive profile of individuals with PTSD may differ between men and women. Further research regarding these potential sex differences in neurocognitive outcomes of PTSD as well as treatment development in addressing these cognitive differences between sexes are needed to address these findings.

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Poster

547. Pain, Headache, Migraine, and Trauma

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Topic: G.06. Post-traumatic Stress Disorder

Support: NSFC Grant 81401477

Title: Meta-analysis reveals trauma-specific gray matter alterations in PTSD

Authors: L. MENG[1], J. JIANG[1], C. JIN[3], J. LIU[1], Y. ZHAO[1], W. WANG[1], *K. LI[2], Q. GONG[1];
[1]West China Hosp., Chengdu, China; [2]West China Hosp., Sichuan, China; [3]Zigong Mental Hlth. Ctr., Zigong, China

Abstract: PURPOSE: Posttraumatic stress disorder (PTSD) is the only major mental disorder with a known cause, i.e., an event that threatens one's physical integrity or that of others. Examples of traumatic events are natural disasters, accidents, combats, childhood abuse, sexual abuse and indirect exposure by learning that a close relative or a friend was exposed to trauma. Previous studies have demonstrated that patients with posttraumatic stress disorder (PTSD) caused by different traumas may show divergence in epidemiology, clinical manifestation and treatment outcome. However, it is still unclear whether this divergence has neuroanatomical correlates in PTSD brains.
METHODS**:** To elucidate the general and trauma-specific cortical morphometric alterations, we performed a quantitative voxel-wise meta-analysis of grey matter (GM) changes in PTSD with different traumas and trauma-exposed controls (TEC) using anisotropic effect-size signed differential mapping (AES-SDM) and its subgroup analysis. Meta-regression was used to explore the effects of demographics and clinical characteristics.
RESULTS: Sixteen studies, consisting of 246 PTSD patients and 347 TECs, met our inclusion criteria. The pooled meta-analysis and subgroup meta-analyses revealed general GM reduction (GMR) foci in the prefrontal-limbic-striatal system of PTSD brains compared with those of TECs. Notably, the GMR patterns are trauma-specific. For PTSD by accidents, GMR foci were found in bilateral anterior cingulate cortex (ACC) and medial prefrontal cortex (mPFC); for PTSD by natural disasters in bilateral mPFC and ACC, the left amygdala and the left hippocampus; while for PTSD by combats, GMR foci were found in the left striatum, the left insula and the left middle temporal gyrus (MTG). Moreover, Clinician-Administered PTSD Scale scores were found significantly associated with GMRs in bilateral ACC and mPFC.
CONCLUSIONS: In summary, this meta-analysis performed a quantitative voxel-wise meta-analysis of GM changes in PTSD with different traumas using AES-SDM. We found that GMR regions were generally located in the prefrontal-limbic-striatal structures. In particular, subgroup

analyses revealed that the GMR patterns were associated with specific trauma categories. It provides further evidences for different brain mechanisms underlying PTSD by different traumas, and suggests that stratified diagnosis and treatment for PTSD patients are necessary in clinics.

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Poster

547. Pain, Headache, Migraine, and Trauma

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Program#/Poster#: 547.08/JJJ37

Topic: G.06. Post-traumatic Stress Disorder

Support: VA 0214BRRC-17

Title: Modulation of signs and symptoms of post traumatic stress disorder and traumatic brain injury by peripheral nerve stimulation

Authors: *D. G. LAMB[1,2], E. S. C. PORGES[2], J. B. WILLIAMSON[1,2];
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Abstract: Post traumatic stress disorder (PTSD) and other forms of emotional dysregulation frequently occur after mild traumatic brain injury (mTBI). mTBI may result in diffuse axonal injury, disrupting limbic and executive networks. While PTSD is often transient, many do not achieve remission, even with the best available treatments. Unfortunately, chronic symptoms and signs of emotional dysregulation decrease quality of life and contribute to major cumulative health risk factors. Current treatment approaches, including psychotherapy and psychiatric medication, only achieve full remission in less than half of treated individuals. Thus, alternate approaches should be considered as primary treatment and rehabilitation approaches or as adjuvants to current best practices. We evaluated the impact of a non-invasive nerve stimulation approach on measures of PTSD symptoms including emotionally modulated autonomic nervous system function in combat veterans with medical record confirmed diagnoses of PTSD with current active symptoms of PTSD and closed-head mTBI, and healthy combat veteran controls. Participants were randomized into stimulation or sham stimulation groups and given an emotionally-modulated startle-blink paradigm and a postural modulated baroreceptor sensitivity and heart-rate variability assessment. We found that the stimulation was well tolerated and resulted in improvements to electrophysiological measures of autonomic nervous system state

and emotionally-modulated startle response, suggesting that this method affects systems underlying emotional dysregulation in this population.

Disclosures: **D.G. Lamb:** None. **E.S.C. Porges:** None. **J.B. Williamson:** None.

Poster

547. Pain, Headache, Migraine, and Trauma

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Topic: G.06. Post-traumatic Stress Disorder

Support: Deutsche Forschungsgemeinschaft (SFB636/C01)

Title: Deficient fear extinction memory in posttraumatic stress disorder

Authors: ***H. FLOR**[1,2], M. WICKING[1], F. STEIGER[1], M. RUTTORF[2], O. GRIMM[1], L. R. SCHAD[2], F. NEES[1];
[1]Central Inst. Mental Hlth. Heidelberg Univ. Mannheim, Mannheim, Germany; [2]Computer-Assisted Clin. Medicine, Med. Fac. Mannheim, Heidelberg Univ., Mannheim, Germany

Abstract: Posttraumatic stress disorder (PTSD) might be maintained by deficient extinction memory. We used a cued fear conditioning design with extinction and a post-extinction phase to provoke the return of fear and examined the role of the interplay of amygdala, hippocampus and prefrontal regions**.** We compared 18 PTSD patients with two healthy control groups: 18 trauma-exposed subjects without PTSD (nonPTSD) and 18 healthy controls (HC) without trauma experience. They underwent a three-day ABC-conditioning procedure in a functional magnetic resonance imaging scanner. Two geometric shapes that served as conditioned stimuli (CS) were presented in the context of virtual reality scenes. Electric painful stimuli were delivered after one of the two shapes (CS+) during acquisition (in context A), while the other (CS-) was never paired with pain. Extinction was performed in context B and extinction memory was tested in a novel context C. The PTSD patients showed significantly higher differential skin conductance responses than the non-PTSD and HC and higher differential amygdala activity than the HC in context C. In addition, elevated arousal to the CS+ during extinction and to the CS- throughout the experiment was present in the PTSD patients but self-reported differential valence or contingency were not different. During extinction recall, differential amygdala activity correlated positively with the intensity of numbing and ventromedial prefrontal cortex activity correlated positively with behavioral avoidance. PTSD patients show heightened return of fear in neural and peripheral measures. In addition, self-reported arousal was high to both danger (CS+) and safety (CS-) cues. These results suggest that a deficient maintenance of extinction and a failure to

identify safety signals might contribute to PTSD symptoms, whereas non-PTSD subjects seem show normal responses.

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Poster

547. Pain, Headache, Migraine, and Trauma

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Program#/Poster#: 547.10/JJJ39

Topic: G.06. Post-traumatic Stress Disorder

Title: Differential expression of long non coding rna gas5 in post traumatic stress disorder

Authors: *G. GUFFANTI[1], A. WINGO[2], T. JOVANOVIC[2], C. NEMEROFF[3], A. MYERS[3], K. J. RESSLER[1];
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Abstract: BACKGROUND: Evidence is accumulating that non-coding RNAs might play arole in GR-mediated regulation of glucocorticoid signaling. lncRNA GAS5 has recently been characterized as a suppressor of transcriptional activity of glucocorticoid responsive genes in mice. Human genetic studies examining stress pathways have mostly focused on the glucocorticoid receptor (GR) and its target genes. Transcriptional and post-transcriptional regulatory function of lncRNAs might explain the missing "link" between the genetic make-up predisposing to PTSD and stress- and trauma-related disorders and GR-related neurobiology in a tissue specific way. We set up to test the hypothesis that lncRNA GAS5 regulation might be perturbed in PTSD. **METHODS:** The sample derives from the larger cohort of the Emergency Department (ED) collaboration for the analysis of the transcriptome of peripheral blood of PTSD patients. PTSD was diagnosed immediately after the trauma and confirmed up to three months later, yielding 23 cases and 23 trauma-exposed controls. Raw paired-end RNA-sequencing was aligned to the hg19 genome build using the Tuxedo pipeline. Further processing to delineate the transcriptional profiles of lncRNA was performed using the ad hoc lncRNA pipeline called PLAR. We tested for statistical significance of observed changes in gene expression between PTSD patients and trauma-exposed controls using the statistical negative binomial model implemented in R-Bioconductor EdgeR.**RESULTS:** We have characterized GAS5 expression profiles in a subset of 46 subjects among cases and controls from the GTP peripheral RNA-seq cohort. Out of the 29 non-redundant isoforms of GAS5, we were able to detect the expression of 12 isoforms. At least two GAS5 isoforms revealed significant down-regulation in PTSD patients compared to trauma-exposed controls ($p < 10\text{-}4$). Based on the information reported in the gene

expression database GTEx, these isoforms seem to be expressed in peripheral blood tissue but do not seem to be expressed in AM nor HC in favor of other isoforms.**CONCLUSIONS:** The results highlight the importance of tissue specificity in the assessment of lncRNA expression profiles, and support the original hypothesis that lncRNA GAS5 plays a role in PTSD, and suggest that lncRNA GAS5 may be differentially expressed in stress-related syndromes compared to traumatized controls. The mechanism by which GAS5 performs its regulatory function remains to be elucidated.

Disclosures: G. Guffanti: None. **A. Wingo:** None. **T. Jovanovic:** None. **C. Nemeroff:** None. **A. Myers:** None. **K.J. Ressler:** None.

Poster

547. Pain, Headache, Migraine, and Trauma

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 547.11/JJJ40

Topic: G.06. Post-traumatic Stress Disorder

Title: Brain structure associated with military deployment and its relation to PTSD symptomatology in a non-clinical population

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Abstract: Research investigating the effects of trauma exposure on brain structure and function in adults has mainly focused on posttraumatic stress disorder (PTSD). The effects of repeated or long-term stress exposure on brain structure, in the absence of psychopathology, have largely remained unexplored.
By assessing 27 healthy combat trauma-exposed individuals by means of whole-brain voxel-based morphometry on 3 T MRI scans, we identified a negative association between duration of military deployment and gray matter volumes in ventromedial prefrontal cortex (vmPFC) and dorsal anterior cingulate cortex (ACC). We also found a negative relationship between deployment-related gray matter volumes and psychological symptoms, but not between military deployment and psychological symptoms.
This is the first whole-brain analysis showing that longer military deployment is associated with smaller regional brain volumes in combat-exposed individuals without PTSD. Notably, the observed grey matter associations resemble those previously observed in PTSD populations, and

concern regions involved in emotional regulation and fear extinction. The current data cannot unambiguously identify the temporal dynamics of these associations but are consistent with the hypothesis that stress exposure leads to alterations in brain structure, resulting in reductions in fear regulation and greater subsequent psychological symptomatology, even in individuals without a clinical diagnosis. These findings have implications on the current dichotomy between clinical and subclinical populations in PTSD neuroimaging research and emphasize the need to incorporate measures of stress exposure when investigating differences between PTSD patients and trauma-exposed controls.

Disclosures: O. Butler: None. **G. Willmund:** None. **P. Zimmermann:** None. **U. Lindenberger:** None. **J. Gallinat:** None. **S. Kühn:** None.

Poster

547. Pain, Headache, Migraine, and Trauma

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Program#/Poster#: 547.12/JJJ41

Topic: G.06. Post-traumatic Stress Disorder

Support: TATRC W81XWH-11-2-0166

Dielmann Family Genetic and Environmental Risk Endowment

Title: Calbrain is reduced in PTSD orbitofrontal cortex in parallel with reductions in dendritic spine density

Authors: *K. A. YOUNG[1], L. D. SELEMON[2], D. A. CRUZ[3], D. E. WILLIAMSON[3]; [1]Psychiatry & Behavioral Sci., Texas A&M HSC / Central Texas VA, Temple, TX; [2]Yale Univ. Sch. of Med., New Haven, CT; [3]Duke Univ. Sch. of Med., Durham, NC

Abstract: The orbitofrontal cortex (OFC, BA11) plays an important role in the management of emotional learning and helps link cognitive processing with visceromotor responding. As outlined by Ongur and Price (2000), the OFC integrates information from the cortex, amygdala and mediodorsal thalamus and provides output to the striatum and brainstem/hypothalamus, modulating the autonomic nervous system and the HPA axis. One of its functions may be to act as a buffer between fear signaling from amygdala-related circuits and regulation of autonomic nervous system (ANS). We previously examined the relationship between dendritic OFC spine densities and FKBP5 gene expression in PTSD and normal controls (n=8/8). ANCOVA revealed that PTSD cases had a significantly elevated density of stubby spines and a trend for a reduction in mushroom spine density, with an inverse correlation between FKBP5 gene expression and

mushroom/overall spine density (Young et al., 2015 PMID 26844242) In the present study, we investigated gene expression of calbrain (CABP1), a calcium channel protein associated with synaptic function. Specifically, calbrain is a calmodulin "antagonist" at the C-terminal domain of L-type calcium channels like CACNA1C. Reductions in calbrain occurred in parallel with reductions in mushroom spine density in PTSD (20-30% reductions in both spine density and calbrain expression). Calbrain may be one of a class of transcripts that are expressed at lower levels because there are fewer mushroom spines in PTSD. Alternatively, it could be expressed at normal levels within existing neurons, but neuron numbers could be reduced in PTSD. Either of these anatomical changes could stem from premature senescence related to ANS and/or HPA axis regulation in PTSD.

Disclosures: K.A. Young: None. **L.D. Selemon:** None. **D.A. Cruz:** None. **D.E. Williamson:** None.

Poster

547. Pain, Headache, Migraine, and Trauma

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Topic: G.06. Post-traumatic Stress Disorder

Support: NIH Grant MH090366-01

NIH Grant MH089704-01

Title: Rumination is associated with PTSD severity, symptom clusters, and self related brain structures

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Abstract: Previous research has associated the occurrence of rumination with PTSD. Additionally, neuroimaging studies have consistently implicated the rostral anterior cingulate (rACC) and posterior cingulate cortex (PCC) in self-related thought, including rumination. Previous functional imaging studies have found abnormal brain activity in these self-related brain regions, rACC and PCC, in PTSD. However, no research to date has examined rumination and structural neuroimaging measures in relation to PTSD severity and symptom clusters. In the current study, we measured rumination and collected structural MRI scans in trauma-exposed (n = 16) and PTSD (n = 73) participants. Compared to trauma-exposed subjects, PTSD participants reported higher levels of rumination ($p < 0.05$). Within the PTSD group, we found a correlation

between rumination and PTSD symptom severity ($r = 0.460$, $p = 0.000$) as well as rumination and all PTSD symptom clusters: re-experiencing, avoidance, and hyperarousal (each $p < 0.01$). We also found a significant correlation between PTSD and right PCC volume as well as a trend-level correlation for right rACC volume and thickness. Together, these results are consistent with previous clinical and neuroimaging studies associating PTSD with rumination, negative self-related thought, and altered activity in self-related brain regions. Future research will be necessary to investigate the relationships among negative self-thought, structure and function of self-related brain regions, and PTSD symptoms and severity after successful treatment.

Disclosures: **T.A. Floyd:** None. **C.L. Philippi:** None. **S.E. Bruce:** None.

Poster

547. Pain, Headache, Migraine, and Trauma

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Program#/Poster#: 547.14/JJJ43

Topic: G.06. Post-traumatic Stress Disorder

Support: W81XWH-08-2-0110 Dept of Defense

Title: Whole-genome epigenetic changes in postmortem human posttraumatic stress disorder brains

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Abstract: Recent evidence from genome-wide association studies coupled with targeted genetic studies has begun to point toward the importance of genetic contributions to posttraumatic stress disorder (PTSD). However few studies to date have examined epigenetic mechanisms, specifically DNA methylation targeting brain regions previously implicated in this debilitating condition. Here we report on a study examining 19 PTSD brains and 38 controls. DNA was isolated from the medial orbital frontal cortex (mOFC) (BA11) and motor cortex (MC) (BA4). DNA methylation of CpG sites across the genome was assessed using the Illumina 450K Methylation BeadChip. Gene set enrichment analysis (GSEA) in mOFC revealed decreased methylation in PTSD for genes enriched in several genetic pathways. These pathways include glutamate receptor activity (e.g. GRIA1 & 2 and GRM1,3-5), glutamate signaling (e.g. GRIA3 & 4), and neuronal differentiation (e.g. YWHAH, APOE and BAI1). Similarly, GSEA in MC revealed PTSD brains to be hypomethylated for genes involved in axonogenesis (e.g. S100B,

AMIGO1, and APOE) and glutamate signaling (e.g. GRIA3 & 4). These results suggest that the hypomethylation of these gene pathways are likely to result in alterations in the expression of these genes and may play a central role in the onset and persistence of PTSD. Moreover, these results add to a growing body of literature implicating changes in the glutamate signaling pathway in PTSD and associated morphological changes in neuronal development.

Disclosures: D.A. Cruz: None. **K.A. Young:** None. **D.E. Williamson:** None.

Poster

547. Pain, Headache, Migraine, and Trauma

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Topic: G.06. Post-traumatic Stress Disorder

Support: VA CDA # IK2 BX002712

American Sleep Medicine Foundation

Portland VA Research Foundation

NARSAD/Brain and Behavior Foundation

Title: Quantitative sleep EEG in a mouse model and in human subjects with post-traumatic stress disorder

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[1]Portland VA Res. Fndn., Portland, OR; [2]North Florida/South Georgia Veterans Hlth. Syst., Gainesville, FL; [3]Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Post-traumatic stress disorder (PTSD) often results in significant sleep disturbances, daytime sleepiness, depressed mood and hypervigilance. Little is known about the interactions between sleep and the other relevant neural circuits involved in chronic stress. We analyzed sleep staging, EEG coherence, and behavior in a mouse model of PTSD (Single Prolonged Stress (SPS), a well-validated protocol which comprises a series of stressors including restraint stress, a group forced swim, exposure to ether, and social isolation for 1 week (n=7-9 per group)). Following SPS, mice underwent fear extinction testing and EEG/EMG signals were recorded and scored as Wake, NREM, and REM sleep. EEG coherence (a normalized value that reflects the degree of coupling between EEG waveforms from 2 skull sites for a given frequency bin) was computed using MATLAB. As expected, SPS mice showed significantly more freezing behavior

during the fear extinction task ($p < 0.05$). In the novel environment task, SPS mice showed significantly more sleep-wake transitions ($p < 0.01$) and a shorter latency to fall asleep ($p < 0.01$) compared to controls. SPS mice showed significantly decreased delta EEG coherence between hemispheres during NREM sleep ($p < 0.05$). Next, human subjects with sleep disturbances were consented during in-lab polysomnography and administered validated questionnaires to assess self-reported sleep and PTSD symptoms. As expected, Veterans with severe PTSD symptoms reported more insomnia and worse functional outcomes from sleep disturbances compared to those with mild or no PTSD symptoms ($p < 0.05$). Similar to mice after SPS, human subjects with PTSD showed significantly decreased delta EEG coherence between right and left hemispheres during NREM sleep ($p < 0.05$). Furthermore, the severity of PTSD symptoms as reported on the PCL-5 scale was significantly negatively associated with NREM delta EEG coherence ($R^2 = 0.78$, $p < 0.001$). In summary, our data suggest that quantitative EEG markers during sleep may represent a useful marker to further query brain circuit dysfunction and individual differences in PTSD symptomatology in both mice and human subjects. Ongoing studies will examine patterns of neural activation across sleep and stress circuits, and relationships between EEG and behavior.

Disclosures: **R.A. Opel:** None. **M. Modarres:** None. **D.J. Akins:** None. **N.N. Kuzma:** None. **M.R. Gieger:** None. **M.M. Lim:** None.

Poster

547. Pain, Headache, Migraine, and Trauma

Location: Halls B-H

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Program#/Poster#: 547.16/JJJ45

Topic: I.05. Biomarker and Drug Discovery

Title: Alpha 7 nicotinic receptor agonist attenuates pain-evoked brain function in an experimental pain fMRI paradigm

Authors: *R. RAJAGOVINDAN, R. CARR, B. HOOKER, C. ZHU, M. SCHRIMPF, L. LEE, J. BEAVER;
AbbVie, North Chicago, IL

Abstract: Pain is a complex percept involving supraspinal processing that is not adequately characterized by simple reflexive preclinical measures of pain and subjective patient reports resulting in poor preclinical-clinical translation. In contrast, functional MRI (fMRI) has emerged as a promising tool to probe pain-related brain function associated with disease and therapeutic intervention in both preclinical and clinical setting. In this study we investigate the effects of an alpha 7 (α_7) nicotinic receptor agonist (ABT-107) on pain-related brain function, previously

shown to be effective in various preclinical models of pain. α_7 receptors are expressed on GABAergic neurons of brain and spinal cord and are implicated in pain processing. Knockout models exhibit hyperalgesia and agonist ligands demonstrate analgesic action. We hypothesized that ABT-107 will attenuate pain-evoked blood oxygen level-dependent (BOLD) fMRI response in brain regions that mediate pain processing underlying its analgesic action. We employed noxious electrical stimulus (NES) evoked pain fMRI paradigm to test this hypothesis. Following a single acute dose of ABT-107 (2 μmol/kg, n=9) or vehicle (n=9), NES was administered to the plantar surface of the paw following a block design in anesthetized animals. T2* weighted fMRI images were acquired using a single-shot GE EPI sequence on a 4.7T scanner (TR=2000ms, TE=12ms, 0.5x0.5x1.25mm, 14 slices). The functional images were motion corrected, brain extracted, high-pass filtered and subject to first-level general linear modeling followed by group-level mixed-effects analysis to estimate the group means and drug-vehicle contrast using FSL software. Correlation between the magnitude of pain-induced response under the drug condition and the plasma drug level was also assessed. ABT-107 attenuated pain-evoked fMRI response in regions such as the anterior cingulate relative to vehicle group. Statistically significant negative correlation between the fMRI response to NES and plasma drug levels were observed in the anterior cingulate, thalamus and somatosensory cortex; key regions involved in pain processing. Thus inhibition of increased neural activity in pain-related brain circuitry may underlie the analgesic effect of α_7 agonists. Furthermore, these findings demonstrate that fMRI provides a sensitive measure of pain-induced changes in brain function, as well as its modulation following therapeutic intervention highlighting its effectiveness as an experimental medicine tool for pain drug development.

Disclosures: **R. Rajagovindan:** A. Employment/Salary (full or part-time): AbbVie. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **R. Carr:** A. Employment/Salary (full or part-time): AbbVie. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **B. Hooker:** A. Employment/Salary (full or part-time): AbbVie. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **C. Zhu:** A. Employment/Salary (full or part-time): AbbVie. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **M. Schrimpf:** A. Employment/Salary (full or part-time): AbbVie. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **L. Lee:** A. Employment/Salary (full or part-time): AbbVie. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie. **J. Beaver:** A. Employment/Salary (full or part-time): AbbVie. C. Other

Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie.

Poster

547. Pain, Headache, Migraine, and Trauma

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 547.17/JJJ46

Topic: I.05. Biomarker and Drug Discovery

Title: Antisense Oligonucleotides can target the key cell populations in the pain system

Authors: *A. MOHAN, B. FITZSIMMONS, H. ZHAO, H. KORDASIEWICZ, E. SWAYZE; Neurodrug discovery, Ionis Pharmaceuticals, Carlsbad, CA

Abstract: Chronic pain remains an unmet clinical need. Antisense oligonucleotides (ASOs) are an RNA therapeutic capable of selectively downregulating target RNAs, and can be used to suppress traditionally "undrugable" targets. ASOs are stable single strands of DNA that bind to their complementary target RNA and direct its catalytic degradation through the action of RNase H, an endogenous enzyme present in most cells. ASOs do not cross the blood brain barrier, but are soluble in cerebrospinal fluid (CSF) and distribute throughout the CNS when delivered to the CSF via intrathecal (IT) or intracerebroventricular (ICV) injection. ASOs delivered IT or ICV suppress target RNA in the pain system including the brain, spinal cord, DRG and trigeminal ganglia. We set out to characterize the cell populations within these tissues that are targetable with ASOs. To that end, we have treated rodents with ASOs to multiple targets and have used both immunofluorescence with a high-throughput fluorescent laser scanning system and cell sorting techniques to quantify the targetability of the various cell populations in the pain system. The populations assessed include, but are not limited to, NeuN, Iba1, GFAP, CGRP, SP, TRPV1, P2X3, IB4, NF200 positive cells. These data provide a deeper understanding of the pattern of ASO-mediated target suppression at a cellular level in the pain system, which allows us to better implement the ASO technology for the treatment of chronic pain.

Disclosures: **A. Mohan:** A. Employment/Salary (full or part-time): IONIS pharmaceuticals. **B. Fitzsimmons:** A. Employment/Salary (full or part-time): IONIS pharmaceuticals. **H. ZHao:** A. Employment/Salary (full or part-time): IONIS pharmaceuticals. **H. Kordasiewicz:** A. Employment/Salary (full or part-time): IONIS pharmaceuticals. **E. Swayze:** A. Employment/Salary (full or part-time): IONIS pharmaceuticals.

Poster

547. Pain, Headache, Migraine, and Trauma

Location: Halls B-H

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Topic: I.05. Biomarker and Drug Discovery

Title: Activators of the two-pore-domain potassium channel TRESK - potential small molecule therapeutics for migraine and neuropathic pain.

Authors: *P. D. WRIGHT[1], C. KETTLEBOROUGH[1], D. TICKLE[1], Z. CADER[2], J. JERMAN[1];
[1]MRC Technol., Stevenage, United Kingdom; [2]Univ. of Oxford, Oxford, United Kingdom

Abstract: TRESK, also known TWIK-related spinal cord potassium channel or $K_{2P}18.1$, is a two-pore-domain potassium channel. TRESK primarily functions to restore membrane potential, allowing the outward flow of potassium ions as the membrane depolarizes. Mutations in the TRESK gene (*KCNK18*) have been linked to familial migraine with aura and TRESK knockdown mice show behavioral evidence of increased pain and sensitivity to stimuli. Relatively restricted protein expression in sensory ganglia suggests a small molecule TRESK activator could act to reduce neuronal excitability and responsiveness, providing targeted therapeutic benefit in both migraine and neuropathic pain. Using baculovirus to deliver the human TRESK gene into mammalian cells a cell-based assay was developed to measure TRESK activity and identify novel activators. This system enabled controlled expression of TRESK in a variety of cellular backgrounds and demonstrated that titratable protein expression of the channel leads to differential channel activity. Using human TRESK expressing U2OS cells we configured a high throughput 384-well plate-based fluorescence screen to identify selective activators of TRESK. The FluxOR Potassium Ion Channel system (Life Technologies, USA), which utilizes thallium as a surrogate of potassium movement, was used to screen approximately 20000 compounds, including a subset of known ion channel pharmacaphores. A number of novel small molecule activators were identified and activity confirmed in concentration-response curve experiments, with sub-µM activity observed for some examples. Preliminary structure-activity relationship (SAR) studies further reinforced focus on a number of chemical sub-series. Selectivity was assessed versus alternative two-pore-domain potassium channels and non-transduced cells and compounds were confirmed as active at both rat and mouse TRESK. Further investigation into the activity and molecular mechanism of activity for these compounds is on-going. Initial screening identified Cloxyquin (5-Chloro-8-ol) as an activator of TRESK (EC_{50} 3.8µM). Cloxyquin was shown to be selective versus the alternative potassium channels TREK, ROMK and hERG. Importantly, Cloxyquin was also shown to activate TRESK in conventional whole-cell electrophysiology experiments, validating the use of a thallium assay in the screening cascade. It is hoped that compounds with appropriate TRESK activity and acceptable physical

chemical properties can be used to further elucidate the role of TRESK in behavioral models of migraine, neuropathic pain and ultimately lead to novel therapeutics.

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Poster

547. Pain, Headache, Migraine, and Trauma

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Topic: I.05. Biomarker and Drug Discovery

Support: These studies were supported by bridging funds provided by the UAMS Department of Pharmacology and Toxicology.

Title: Characterization of G-protein biased CB1 agonists reveals potential for development of functionally selective cannabinoids with improved therapeutics

Authors: *B. M. FORD, S. TAI, L. N. FRANKS, W. E. FANTEGROSSI, P. L. PRATHER; Pharmacol. and Interdisciplinary Toxicology, Univ. of Arkansas For Med. Sci., Little Rock, AR

Abstract: The cannabinoid subtype 1 receptor (CB1R) is ubiquitously expressed in the CNS and serves as a target for modulating nociception in acute and neuropathic pain by endogenous ligands as well as synthetic cannabinoids. Unfortunately, acute use of cannabinoids acting at CB1 receptors also produces unwanted psychotropic effects, and chronic administration results in development of tolerance and dependence. Studies in β- arrestin knockout mice suggest that interaction of certain GPCRs, including μ-, δ-, κ- opioid and CB1-receptors, with β-arrestins might be responsible for several adverse effects produced by agonists acting at these receptors. Indeed, agonists that bias opioid receptor activation toward G-protein, relative to β-arrestin signaling, produce less severe adverse effects. These observations indicate that therapeutic utility of analgesics acting at CB1 receptors might be improved by development of G-protein biased CB1 agonists. Our laboratory recently reported a novel class of indole quinuclidine (IQD) compounds that bind cannabinoid receptors with relatively high affinity and act with varying intrinsic activity. The purpose of this study was to determine whether agonists in this novel cannabinoid class exhibit ligand bias at CB1 receptors. Our studies found that a novel IQD-derived CB1 receptor agonist PNR-4-20 elicits robust G-protein signaling (as measured by G-protein activation and inhibition of cAMP accumulation), with transduction ratios similar to CP-55,940. In marked contrast, PNR-4-20 produces little to no β-arrestin recruitment. Quantitative calculation of bias factors indicate that PNR-4-20 exhibits from 55- to 210-fold bias for G-

protein, compared to β-arrestin signaling (when compared to G-protein activation or inhibition of cAMP accumulation, respectively). Importantly, as expected due to reduced β-arrestin recruitment, chronic exposure of cells to PNR-4-20 results in significantly less desensitization and down-regulation of CB1 receptors compared to similar treatment with CP-55,940. Finally, PNR-4-20 (i.p.) is active in the cannabinoid tetrad in mice and studies of a structurally similar analog PNR- 4-02 is also a highly G-protein biased CB1 agonist. It is predicted that, similar to recently reported data for biased opioid agonists, cannabinoid agonists that bias CB1 receptor activation toward G-protein, relative to β-arrestin signaling, will produce fewer and less severe adverse effects both acutely and chronically.

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Poster

547. Pain, Headache, Migraine, and Trauma

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 547.20/JJJ49

Topic: I.05. Biomarker and Drug Discovery

Support: JSPS KAKENHI 26670290

Title: Application of calibrated forceps for evaluating antinociceptive effect induced by short-acting analgesic in mice

Authors: E. INOUE[1], T. KUWAKI[1], *H. KASHIWADANI[2];
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[2]Kagoshima Univ., Kagoshima, Japan

Abstract: In nociceptive information processing, subpopulations of peripheral nociceptors and central nociceptive neurons are involved depending on nociceptive modalities. Therefore, the pain-relieving effects of analgesics could vary among those modalities. Though various sources of stimulation can evoke nociception, the thermal, the chemical, and the mechanical modalities are frequently used to evaluate the analgesics. In the past, several standardized nociceptive tests have been developed with respect to the modality: the von Frey test, the Randall-Selitto test, and the calibrated forceps test for mechanical; the hot/cold plate test, the radiant heat test, and the tail flick test for thermal; and the formalin test for chemical nociception. Of those tests, the von Frey test has been most frequently applied to examine mechanical nociception in mice because the Randall-Selitto and calibrated forceps tests were developed for rats. The von Frey test, however, takes a long time to determine the nociceptive threshold so it is inadequate to evaluate the rapid

change of the threshold induced by short-acting analgesics. In this study, we evaluated the capability of the calibrated forceps test to measure the mechanical nociceptive threshold in the tail of mice. Daily measurements of the threshold revealed that the device obtained stable and reliable results. Furthermore, repeated measurements with 5 minute intervals detected the rapid change of the threshold induced by subcutaneous injection of remifentanil, a short-acting μ-receptor agonist. In the meeting, we will also discuss time course data pertaining to odor-induced analgesia in mice.

Disclosures: **E. Inoue:** None. **T. Kuwaki:** None. **H. Kashiwadani:** None.

Poster

547. Pain, Headache, Migraine, and Trauma

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Program#/Poster#: 547.21/JJJ50

Topic: I.05. Biomarker and Drug Discovery

Support: NCCIH AT007987

Title: Limbic brain asymmetry predicts placebo response propensity in chronic back pain

Authors: ***T. B. ABDULLAH**, S. BERGER, E. VACHON-PRESSEAU, A. APKARIAN, T. SCHNITZER;
Northwestern Univ., Chicago, IL

Abstract: The limbic system is involved in emotional regulation, cognition, memory formation, and reward processing. It is comprised of cortical and subcortical regions including specifically the prefrontal cortex, amygdala, hippocampus, and nucleus accumbens. Here we investigate of the relationship between subcortical volume differences and placebo response propensity in chronic back pain patients (CBP). We collected high-resolution anatomical images from 67 CBP patients who were randomized into one of three treatment arms: placebo (n=43), no treatment (n=20), and positive control (n=4). Participants were enrolled for a total of eight weeks and completed 2 two-week treatment periods each followed by a one-week washout. Additionally, participants were asked to continuously monitor their pain and mood twice a day using a smartphone application. Here, we analyzed data from the first brain scans collected before any treatment was administered, to unravel anatomical biomarkers that a priori predispose subjects for placebo response. We extracted subcortical structural volume measurements using FSL-First and Freesurfer. We examined the ratio of right to left subcortical volume measurements. This measurement was created by summing the amygdala, hippocampus, and nucleus accumbens volumes for each hemisphere, and taking the ratio of right/left subcortical volume for each

person. Participants were classified as responders based on a permutation test between pain ratings acquired during baseline and during each of the treatment periods. The null hypothesis was generated by randomly resampling the distribution of pain ratings 10,000 times and was rejected at $p < 0.05$. Based on this criterion, we obtained 24 responders and 19 non-responders to placebo. On average, placebo responders showed a nearly proportional symmetry, while non-responders had a smaller right limbic volume; those individuals allocated to no-treatment had ratios in between these two groups ($F(2,60) = 4.12$, $p = 0.028$; Responders = 1.01, Non-Responders = 0.96, No treatment = 0.99). Volumetric outputs by FSL-FIRST showed significant results, while Freesurfer did not significantly differ between the groups, but showed a similar trend. These results indicate significant anatomical differences that are present prior to treatment, implying that limbic structural neuroanatomy may contribute to a predisposition to respond to placebo in individuals with CBP. Funded by: NIH (NCCIH) AT007987

Disclosures: **T.B. Abdullah:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine, Department of Physiology. **S. Berger:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine, Department of Physiology. **E. Vachon-Presseau:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine, Department of Physiology. **A. Apkarian:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine, Department of Physiology. **T. Schnitzer:** A. Employment/Salary (full or part-time): Northwestern University Feinberg School of Medicine, Department of Physical Medicine and Rehabilitation and Medicine-Rheumatology.

Poster

547. Pain, Headache, Migraine, and Trauma

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 547.22/JJJ51

Topic: I.05. Biomarker and Drug Discovery

Support: VA RR&D SPIRE AWARD

Title: rTMS induced supraspinal functional changes in patients with MTBI related headache

Authors: *A. KHALAF[1], M. LIM[1], E. YANG[1], V. METZGER-SMITH[2], J. CORDERO[1], Y. HE[1], L. LIN[2], D. D. SONG[1], R. R. LEE[1], G. R. POLSTON[1], A. TSAI[2], A. LEUNG[1]; [1]UCSD, La Jolla, CA; [2]VA San Diego Healthcare Syst., La Jolla, CA

Abstract: Introduction:
Repetitive transcranial magnetic stimulation (rTMS) exists as a feasible non-invasive and low

cost therapeutic tool for those suffering from mild traumatic brain injury related headaches (MTBI-HA)[1; 2]. This study aims to assess the effect of rTMS in improving supraspinal pain processing and modulatory functions in patients with MTBI-HA.

Materials and Methods: Veterans with MTBI-HA were randomized to receive four treatments (>24 and <72 hours apart) of either Real or Sham rTMS at the left dorsolateral prefrontal cortex (LDLPFC) with neuronavigation guidance. Pre- and One-Week Post-treatment functional magnetic resonance imaging (fMRI) assessments were performed in a GE 3.0T 750 MRI scanner with T2*- weighted EPI-sequence (TE=30 ms, TR=2.0s, α=90°, TH=4mm, 32 slices, FOV=220x220 mm^2, MA=64x64) while intermittent 6 seconds of subject specific heat pain was delivered via fMRI-compatible Peltier probe at the medial aspect of the left mid-calf with various intervals (20-40 seconds) of a baseline temperature of 32°C. Heat pain stimulation was repeated 20 times to complete the session [3].

Results: Pre-treatment between-group (Real minus Sham) random effect analyses showed no significant difference. In the Real Post-minus-Pre treatment between group analysis, a significant ($P<0.01$, cluster threshold>150) deactivation in the rostral anterior cingulate cortex (rACC) was noted. In Sham Post-minus-Pre treatment between group analysis, deactivation ($P<0.01$, cluster threshold>150) was noted in the secondary somatosensory cortex (SSC2), premotor, and temporal cortices. In Real-minus-Sham Post-treatment between group analysis, significant ($P<0.01$, cluster threshold>150) deactivation was noted in the basal ganglia, rACC, ventral cingulate cortex, and SSC2.

Conclusion: In patients with MTBI-HA, rTMS at the LDLPFC appears to diminish activity in supraspinal regions involved in encoding acute and chronic pain. The rTMS treatment most notably appeared to decrease activation in the rACC, which is thought to underlie the affective component of pain experience, and the SSC2, which is involved in somatosensory response to pain. Correlation with treatment related headache relief benefit is to be established.

[1] Leung A, Fallah A, Shukla S. TMS in alleviating post-traumatic peripheral neuropathic pain States: a case series. Pain Med 2014;15(7):1196-1199.

[2] Leung A, Fallah A, Shukla S, Lin L, Tsia A, Song D, Polston G, Lee R. rTMS in Alleviating MTBI-HA - A Case Series. Pain Physician 2016;19(2):E347-354.

[3] Leung A, Shukla S, Li E, Duann JR, Yaksh T. Supraspinal characterization of the thermal grill illusion with fMRI. Mol Pain 2014;10:18.

Disclosures: **A. Khalaf:** None. **M. Lim:** None. **E. Yang:** None. **V. Metzger-Smith:** None. **J. Cordero:** None. **Y. He:** None. **L. Lin:** None. **D.D. Song:** None. **R.R. Lee:** None. **G.R. Polston:** None. **A. Tsai:** None. **A. Leung:** None.

Poster

547. Pain, Headache, Migraine, and Trauma

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 547.23/JJJ52

Topic: I.05. Biomarker and Drug Discovery

Title: Fibromyalgia and microglial TNF-α: Translational research using human blood induced microglia-like cells.

Authors: *M. OHGIDANI[1], T. A. KATO[1], M. HOSOI[1], M. TSUDA[1], K. HAYAKAWA[1], C. HAYAKI[1], R. IWAKI[1], R. HASHIMOTO[2], K. INOUE[1], N. SUDO[1], S. KANBA[1];
[1]Kyushu Univ., Fukuoka, Japan; [2]Osaka Univ., Osaka, Japan

Abstract: Background: Fibromyalgia is a refractory disease characterized by chronic pain, the cause of which has not yet been elucidated due to its complex pathology. Recently, activation of immune cells in the brain called microglia has attracted attention as a potential underlying pathological mechanism in chronic pain. Until recently, however, technological and ethical considerations have limited the ability to conduct research using human microglia.
Methods: We have developed a technique to create human-induced microglia-like (iMG) cells from human peripheral blood monocytes. This study investigated activation of iMG cells in patients with fibromyalgia at the cellular level. iMG cells were created from 14 patients with fibromyalgia and 10 healthy individuals, and analyzed.
Results: No significant difference in phagocytic capacity or cytokine response associated with phagocytosis was observed between iMG cells derived from healthy participants and patients with fibromyalgia. Interestingly, however, the expression of the cytokine tumor necrosis factor-α (TNF-α) at mRNA and protein levels significantly increased in ATP-stimulated iMG cells from patients with fibromyalgia compared to cells from healthy individuals. Furthermore, there was significant correlations between ATP-induced upregulation of TNF-α expression and clinical parameters of subjective pain and other mental manifestations of fibromyalgia.
Conclusions: These findings suggest that the microglia in patients with fibromyalgia are hypersensitive to ATP. TNF-α from microglia may be a key factor underlying the complex pathology of fibromyalgia.

Disclosures: M. Ohgidani: None. **T.A. Kato:** None. **M. Hosoi:** None. **M. Tsuda:** None. **K. Hayakawa:** None. **C. Hayaki:** None. **R. Iwaki:** None. **R. Hashimoto:** None. **K. inoue:** None. **N. Sudo:** None. **S. Kanba:** None.

Poster

548. Drug Addiction: Translational Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 548.01/JJJ53

Topic: G.08. Drugs of Abuse and Addiction

Support: Mayo Clinic S C Johnson Genomics of Addiction Program

Ulm Foundation

David Lehr Research Award from American Society for Pharmacology and Experimental Therapeutics

NIAAA (AA018779)

Title: Discovery of biomarkers associated with acamprosate treatment response by untargeted metabolomics

Authors: *M. HO[1], D. TUMPA[2], D. J. HINTON[1], S. CHOI[1], V. M. KARPYAK[3], M. A. FRYE[3], J. M. BIERNACKA[4], D.-S. CHOI[1];
[1]Mol. Pharmacol. and Exptl. Therapeut., [2]Metabolomics Resource Core, [3]Psychiatry and Psychology, [4]Hlth. Sci. Res., Mayo Clin., Rochester, MN

Abstract: Metabolomic biomarkers will increase treatment response and provide novel insights for new drug development for alcohol use disorder (AUD). Using acamprosate as a probe medication, we aims to discover metabolomic biomarkers to predict acamprosate treatment outcome and to uncover metabolic pathways associated with positive treatment outcome of acamprosate. We collected serum samples from 84 alcohol-dependent subjects before and after a 3-month acamprosate treatment and identified the biomarkers using ultra high performance liquid chromatography (UPLC) and time-of-flight mass spectrometry (ToF/MS). Subjects were classified as responders if they had maintained complete abstinence during the 3-month treatment, or else as non-responders. Comparing relative levels of metabolites between response groups, 48 metabolites were significantly different at baseline (unpaired t test $q<0.001$). These metabolites might contain metabolomic biomarkers for predicting acamprosate treatment outcome. Comparing relative levels of metabolites at 3-month to baseline, responders showed significant changes in 100 metabolites whereas non-responders only had changes in 16 metabolites (paired t test: $q<0.05$). Interestingly, pathway analysis on the 95 metabolites revealed that caffeine metabolism was significantly enriched ($q<0.05$) in the responders. Thus, we examined the effects of caffeine in combination of acamprosate on ethanol drinking in ethanol-dependent mice. Co-administration (i.p) of caffeine (30mg/kg) and acamprosate (400mg/kg) reduced ethanol preference and increased water consumption in limited access two-bottle choice

drinking (one-way ANOVA followed by Tukey posthoc $p<0.05$), but did not significantly affect locomotor activity as in sole caffeine administration. In conclusion, we have identified a panel of serum metabolites that might predict acamprosate treatment response. We also discovered that an enhanced caffeine metabolism might contribute to positive acamprosate treatment response, ,suggesting that caffeine could potentially be an adjunct to enhance antidipsotropic property of acamprosate.

Disclosures: **M. Ho:** None. **D. Tumpa:** None. **D.J. Hinton:** None. **S. Choi:** None. **V.M. Karpyak:** None. **M.A. Frye:** None. **J.M. Biernacka:** None. **D. Choi:** None.

Poster

548. Drug Addiction: Translational Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 548.02/JJJ54

Topic: G.08. Drugs of Abuse and Addiction

Support: Stanford Neuroscience Institute NeuroChoice Initiative

Title: Cue reactivity selectively differentiates stimulant-dependent patients and controls

Authors: ***K. HENNIGAN**[1], E. L. S. JENSEN[1], L. NGUYEN[3], K. HUMPHREYS[2], B. KNUTSON[1];
[1]Psychology, [2]Psychiatry, Stanford Univ., Stanford, CA; [3]VA Palo Alto Hlth. Care Syst., Palo Alto, CA

Abstract: Patients with dependency on stimulant drugs such as cocaine and methamphetamine, which exert their reinforcing effects by acting on mesolimbic dopamine circuitry, are particularly susceptible to relapse, yet little is known about which factors confer risk for relapse. We compared neural and behavioral responses to stimulant and other appetitive cues in stimulant-dependent patients (n=8) and healthy volunteers (controls; n=26) as they underwent functional magnetic resonance imaging (FMRI). During a novel cue reactivity task, subjects viewed distinct shapes which preceded images of stimulants (drug cues), alcohol (alcohol control), food (appetitive control), and office supplies (neutral control). As predicted, stimulant-dependent patients exhibited increased activity in response to drug versus neutral control cues in motivational circuitry (i.e., ventral tegmental area, ventral striatum), but controls did not. Both groups, however, showed responses in motivational circuitry to appetitive control cues. Together, these findings suggest that the impact of stimulant dependence on mesolimbic responses is specific to drug cues, rather than all appetitive cues, or other more general processing demands. The selectivity of this group difference suggests that brain activity in

response to drug cues may provide a useful candidate marker for forecasting relapse in the context of stimulant dependence.

Disclosures: **K. Hennigan:** None. **E.L.S. Jensen:** None. **L. Nguyen:** None. **K. Humphreys:** None. **B. Knutson:** None.

Poster

548. Drug Addiction: Translational Studies

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Program#/Poster#: 548.03/JJJ55

Topic: G.08. Drugs of Abuse and Addiction

Support: FRM Grant

Title: Deep brain stimulation of the rat's subthalamic nucleus reduces cocaine-induced addiction-related behaviors

Authors: ***M. DEGOULET**[1], A. TIRAN-CAPPELLO[1], C. BAUNEZ[1], Y. PELLOUX[2]; [1]Inst. de Neurosci. de La Timone, CNRS, Marseille, France; [2]Natl. institute on Drug Abuse IRP, Baltimore, MD

Abstract: Resurgence of surgical techniques such as deep brain stimulation (DBS) for the treatment of psychiatric diseases has recently opened new therapeutic perspectives for the treatment of addiction, since no medication is currently truly effective at treating addicts. Indeed, high frequency stimulation (HFS, ~130Hz) of the subthalamic nucleus (STN), a part of the basal ganglia, reduces the motivation to take cocaine in rats, suggesting a critical contribution of the STN in addiction processes. To further characterize its involvement in cocaine addiction, we tested the effect of STN DBS, applied either at high or low frequency (LFS, ~30Hz), on the loss of control over drug intake and compulsive drug seeking, two key features of drug addiction. After being trained to self-administer cocaine under a fixed, or a seeking/taking, schedule of reinforcement, rats were given the opportunity to escalate their intake across multiple sessions of daily long access (6h per day) to cocaine. During escalation, we recorded STN local field potentials in order to monitor STN oscillations during cocaine intake. Then, some animals were returned to the seeking/taking task in which seeking responses were associated to intermittent punishment (electric foot shock) while others observed a 6-weeks forced withdrawal period before being re-exposed to the daily long access sessions. Although acute (6h) STN HFS prevented the escalation of cocaine intake, both acute and chronic (24h) STN HFS were ineffective on cocaine intake when applied after escalation. However, STN HFS reduced drug intake after extended withdrawal, while non-stimulated animals quickly recovered their pre-

withdrawal cocaine consumption. Interestingly, a progressive increase in the STN low (~6-12Hz), but not high, frequency oscillations was observed during cocaine escalation, but only in compulsive animals exhibiting resistance to punishment afterwards. Finally, LFS, but not HFS, of the STN tends to reduce persistent cocaine seeking observed in compulsive rats. Thus, our results evidence the STN as a critical locus for the development and expression of addiction-related behaviors and further highlight that manipulating STN activity with DBS might normalize important features of cocaine addiction. Overall, these data strengthen the emerging view that STN DBS represent a promising alternative in the treatment of drug addiction.

Disclosures: M. Degoulet: None. **A. Tiran-Cappello:** None. **C. Baunez:** None. **Y. Pelloux:** None.

Poster

548. Drug Addiction: Translational Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 548.04/JJJ56

Topic: G.08. Drugs of Abuse and Addiction

Support: FRM

Fondation de l'Avenir

Title: Deep brain stimulation of the subthalamic nucleus reduces motivation for cocaine while increasing that for apple sauce in the monkey

Authors: *S. RAVEL[1], J. NACEF[1], J.-L. ANTON[1], I. BALANSARD[1], P.-Y. BORIUS[2], C. W. BRADBERRY[3], R. DESBRIÈRE[4], A. EUSEBIO[1], B. NAZARIAN[1], L. RENAUD[1], J.-M. RÉGIS[2], C. BAUNEZ[1];
[1]Inst. De Neurosciences De La Timone, CNRS & AMU UMR 7289, Marseille, France; [2]Gamma Unit, Hop. de la Timone, AP-HM, Marseille, France; [3]NIDA, Baltimore, MD; [4]Hop. Saint-Joseph, Marseille, France

Abstract: There is currently no pharmacological treatment for cocaine addiction, therefore it's important to look for alternative treatment strategies. One possibility could be a surgical approach. Indeed, it has been shown in the rat that the inactivation of the subthalamic nucleus (STN), by either lesions or high frequency Deep Brain Stimulation (DBS), reduces motivation for cocaine while increasing motivation for food. It has thus been suggested that STN high frequency DBS could be a good strategy to treat cocaine addiction. Before testing in human addicts, the aim of the present study was to validate this hypothesis in non-human primates. We

have trained two monkeys to work under various schedules of reinforcement (Fixed Ratio 15 (FR15) and Progressive Ratio (PR)) for either apple sauce or cocaine (intraveinous 0.1 mg/kg/injection). After stabilisation of performances, electrodes have been implanted bilaterally in the STN, and chronic stimulation has been further applied (130 Hz, 2V). All conditions (apple sauce-stimulation ON, apple sauce-stimulation OFF, cocaine-stimulation ON, cocaine-stimulation OFF) have been tested in alternance. Results have first shown that the level of motivation was higher for cocaine than for apple sauce before stimulation. Then, after STN DBS, the motivation for apple sauce was significantly increased while that for cocaine was significantly decreased. These results confirm the opposite effect of STN DBS on motivation that has been previously demonstrated in rats. Since decreasing the motivation for the drug, without diminishing other forms of motivation is the goal for a possible treatment of cocaine addiction, STN DBS may thus be the appropriate strategy.

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Poster

548. Drug Addiction: Translational Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: A*MIDEX PR2I program grant

CNRS

AMU

Title: Lesions of the Subthalamic nucleus potentiate the beneficial effect of stranger observer on cocaine self-administration

Authors: ***C. BAUNEZ**[1], E. GIORLA[1], C. MONTANARI[1], K. DAVRANCHE[2], C. MANRIQUE[3], P. HUGUET[2], Y. PELLOUX[1];
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Abstract: Among the global social context in drug addiction, proximal social factors (PSF, i.e those surrounding the drug exposure, such as the presence of a peer) have been shown to affect

drug self-administration, but neurobiological basis of such processes still remain unclear. Here, we focused our interest on the Subthalamic Nucleus (STN), a brain structure which lesion reduces affective responses and motivation for cocaine. Here, we first aimed at determining the involvement of the STN in rewarding properties of social interactions in adult rats (not socially isolated). We then tested whether or not PSF's influence could be modulated by STN lesions on cocaine self-administration. To address the issue regarding the rewarding properties of social interactions in adults, we used a conditioned place preference procedure in which one compartment was associated with the presence of the cagemate, while the other compartment was associated with loneliness. In the self-administration procedure, we have used cages in which rats (sham controls or STN lesioned) could self-administer cocaine, while a peer (familiar or stranger) observed them through a grid allowing communication. The ultrasonic vocalizations (USVs) emitted by both rats were thus recorded. We first found that, in STN lesioned rats, whatever the status, the presence of the peer is rewarding. In contrast, for sham rats, the presence of the cagemate is only rewarding for the dominant individuals. In the second experiment, when the rats are alone in the self-administration chamber, sham and STN rats emit equivalent level of USV. The presence of the familiar observer has no effect on cocaine intake but increases the number of USV emitted in sham rats, while a stranger peer induces a decreased of both consumption and USV. For STN lesioned rats, interestingly, the familiar peer induces a reduced level of both cocaine intake and USV. The stranger observer induces a further decreased intake, but increased number of USV emitted. These results show that STN is involved in the neurobiological basis of the PSF' influence on drug consumption. STN lesions shift the effect of a stranger peer to the familiar one, suggesting that the beneficial influence of a stranger observer can be obtained even in presence of a familiar peer, resulting in a dramatic decrease in drug intake. This makes the STN even more promising as a therapeutic target for drug addiction.

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Poster

548. Drug Addiction: Translational Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: Neurosurgery Research and Education Foundation Award 2014-15

2014 Grant-In-Aid from Louisiana State University

Title: Repeated electrical stimulation of medial prefrontal cortex reduces intravenous methamphetamine self-administration in wistar rats.

Authors: V. BATRA, G. GUERIN, N. GOEDERS, *J. WILDEN;
LSU Shreveport, Shreveport, LA

Abstract: INTRODUCTION: Methamphetamine abuse is a global health problem with no proven treatment. Chronic drug use causes neuroadaptations in reward circuitry, including the nucleus accumbens (Acb), ventral tegmental area (VTA), and medial prefrontal cortex (mPFC). We previously demonstrated that daily, intermittent Acb electrical stimulation reduces intravenous methamphetamine self-administration. The main objective of this study was to determine the effect of repeated electrical stimulation of mPFC on intravenous methamphetamine self-administration. METHODS: Rats implanted with IV catheters and bilateral mPFC electrodes (N=14) underwent daily 2-hour sessions in two-lever (active/IV methamphetamine; inactive/no reward) operant chambers to establish methamphetamine self-administration. After stable responding developed, 45 minutes of treatment with electrical stimulation (100 Hz, 50 pulse train every 2s, 200 micro amp) was administered in a separate environment immediately prior to the daily operant sessions for 4 consecutive days. Following each stimulation session, subjects were disconnected from the stimulation apparatus and moved into the operant chambers. RESULTS: There was a significant decrease in total 2-hour methamphetamine intake on days 2, 3, and 4 with an average reduction of 52% ($P<0.05$). Despite similar histology/electrode function, rats demonstrated a clear *bimodal response* to stimulation. Treatment "non-responders" (N=5; 35%) overall maintained methamphetamine intake while "responders" reduced intake by 83% (N=9; 65%) and demonstrated a prolonged effect by failing to return to baseline levels post-treatment. CONCLUSIONS: Repeated electrical stimulation of mPFC decreased intravenous methamphetamine self-administration on 3 of 4 treatment days. The mPFC is a key neuroanatomical substrate for methamphetamine reinforcement. This region may be a promising target for neuromodulatory intervention in intractable cases of stimulant addiction. Identification of biomarkers underlying stimulation responsiveness could help identify the optimal population for these therapies.

Disclosures: V. Batra: None. **G. Guerin:** None. **N. Goeders:** None. **J. Wilden:** None.

Poster

548. Drug Addiction: Translational Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R15DA038295

Title: Role of training dose in the discriminable stimulus effects of 4-methylmethcathinone (4-MMC) in male Sprague-Dawley rats

Authors: M. D. BERQUIST, II, N. A. THOMPSON, *L. E. BAKER;
Psychol Dept, Western Michigan Univ., Kalamazoo, MI

Abstract: Recreational abuse of synthetic cathinones (aka "bath salts") has recently become a popular alternative to other commonly abused psychostimulant drugs. Preclinical psychopharmacology investigations have characterized the neurochemical and behavioral profile of 4-methylmethcathinone (4-MMC), one of the common constituents of psychoactive "bath salts". Neurochemical and electrophysiological studies indicate 4-MMC is a substrate at monoamine transporters that increases extracellular dopamine and serotonin, similar to the amphetamines and MDMA. Consistent with its neurochemical profile, 4-MMC has a high abuse liability as indicated by evidence of self-administration by nonhumans and self-reports by humans. Only a few published studies have implemented drug discrimination procedures to characterize the interoceptive stimulus effects of 4-MMC. Whereas the drug discrimination paradigm is predictive of the pharmacological actions contributing to these effects, it is considered a highly informative *in vivo* drug-detection assay. The current study implemented drug discrimination methods to train 16 male Sprague-Dawley rats to discriminate either 1 mg/kg (n=8) or 3 mg/kg (n=8) 4-MMC from saline. Once adequate discrimination was observed in each training group, substitution tests were conducted with substances that function as dopamine (DA) releasers (*d*-amphetamine, (+)-methamphetamine), DA transport inhibitors (MDPV, cocaine), or a serotonin (5-HT) releaser (MDMA). Stimulus control was established in fewer sessions and more reliably maintained in the subjects trained with 3 mg/kg compared to those trained with 1 mg/kg 4-MMC. Furthermore, all of the aforementioned test compounds produced full substitution in 1 mg/kg 4-MMC-trained rats. However, only MDMA produced full substitution in rats trained to discriminate 3 mg/kg 4-MMC, while all other compounds produced only partial substitution at doses that significantly reduced response rate. These preliminary findings indicate the relative contribution of DA and 5-HT to the interoceptive stimulus effects of 4-MMC are dose-dependent. Further experiments with DA and 5-HT selective antagonists are in progress. This study expands on current knowledge regarding the psychopharmacology of 4-MMC and may further assist in characterizing its subjective effects in habitual users.

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Poster

548. Drug Addiction: Translational Studies

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Program#/Poster#: 548.08/JJJ60

Topic: G.08. Drugs of Abuse and Addiction

Support: NIEHS T32ES007254

Mitchell Center for Neurodegenerative Diseases, UTMB

Center for Addiction Research, UTMB

Title: PPARgamma agonism to treat white matter damage in cocaine use disorder

Authors: *A. DIMET[1], L. DENNER[1], W. R. MILLER[1], K. CUNNINGHAM[1], M. J. HUENTELMAN[2], S. LANE[3], K. DINELEY[1];
[1]Univ. of Texas Med. Br. at Galveston, Galveston, TX; [2]Translational Genomics Res. Inst., Phoenix, AZ; [3]Univ. of Texas Hlth. Med. Sch., Houston, TX

Abstract: Cocaine use disorder elicits both behavioral and structural changes in the brain. Behaviorally, it is characterized by increased impulsivity, impaired decision-making, and increased reactivity to cocaine-paired cues ("cue reactivity"). Structurally, it is characterized by altered white matter (WM) integrity, implying lost fidelity in the tracts that interconnect the grey matter structures thought to underlie the behavioral changes typical of cocaine use disorder. Rat models of the disorder display both phenotypes. Cocaine craving and relapse behaviors can be modeled in rodents by performing a cue reactivity test following forced abstinence (FA) from chronic cocaine self-administration (SA). We discovered that the FDA-approved peroxisome proliferator-activated receptor γ (PPARγ) agonist pioglitazone (PIO, Actos™) attenuates cue reactivity in rats when PIO is given during FA from cocaine SA, *demonstrating the translational potential of PIO*. Importantly, this decreased cue reactivity is reversed by administration of the PPARγ *antagonist* GW9662 prior to the cue reactivity test. Thus, ***we hypothesize*** that PPARγ agonism counteracts the cocaine-mediated damage underlying cue reactivity through the induction of markers for functional and structural integrity in WM. With RNA-Seq we determined the gene expression profile of rats which received cocaine then FA, rats which received cocaine then PIO during FA, and rats which received cocaine then PIO during FA and GW9662 prior to the cue reactivity test. Using Ingenuity® Pathway Analysis we created functional networks of genes regulated by PPARγ agonism, and these networks have supported our hypothesis and implicated a role for several genes important to the functional and structural integrity of WM. Using Protein Simple Wes™ technology, we are now investigating the expression of proteins important to WM integrity which are affected by chronic cocaine use and regulated by PPARγ and/or phosphorylated extracellular signal-regulated kinase, a protein which

we have found to be in complex with PPARγ during memory consolidation and thus a potential co-regulator. Current results support that the expression of some proteins important to WM integrity is modulated by PPARγ agonism, while others' expression is modulated by cocaine but not by PPARγ agonism. In conclusion, our work reveals that the attenuation of cue reactivity through PPARγ agonism may be achieved, at least in part, through modulation of the expression of proteins important to WM integrity. Further studies are needed to determine if the expression of these proteins plays a direct role in the behavioral phenotypes of the cocaine use disorder model.

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Poster

548. Drug Addiction: Translational Studies

Location: Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: R01 DA039139-01

P30 DA013429-16

Title: The glutamate carboxypeptidase II (GCPII) inhibitor 2-PMPA reduces the rewarding properties of 3,4-methylenedioxypyrovalerone (MDPV) in rats: the role of N-acetylaspartylglutamate (NAAG)

Authors: *C. HICKS[1,2], R. A. GREGG[1,2], S. U. NAYAK[1,2], C. S. TALLARIDA[1,2], A. B. REITZ[3], G. R. SMITH[3], S. M. RAWLS[1,2];
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Abstract: 3,4-methylenedioxypyrovalerone (MDPV) is a commonly abused constituent of the synthetic cathinone class of illicit psychoactive substances ("Bath salts"). We recently showed that MDPV withdrawal alters glutamate (GLU) homeostasis in corticolimbic regions via downregulation of the glutamate transporter subtype 1 (GLT-1), and that treatment with a GLT-1 activator attenuates the rewarding effects of MDPV. Another important regulator of GLU transmission involves presynaptic metabotropic glutamate 2 and 3 receptors (mGluR2 and mGluR3) that act to prevent excessive synaptic GLU release. The neuropeptide N-

acetylaspartylglutamate (NAAG) is an endogenous agonist at mGluR2/3s that is inactivated by the glutamate carboxypeptidase II (GCPII) enzyme. Interestingly, GCPII inhibitors, and NAAG itself, attenuate a number of cocaine addiction-related behaviors in rodent models, thereby implicating the NAAG-GCPII signaling pathway in psychostimulant addiction. We hypothesized that administration of a GCPII inhibitor (2-(phosphonomethyl)-pentanedioic acid; 2-PMPA), or NAAG itself, would reduce the rewarding properties of MDPV in rats. Results showed that withdrawal from repeated MDPV administration significantly reduced GCPII protein expression in the prefrontal cortex, but not in the nucleus accumbens or striatum. Systemic injection of 2-PMPA (100 mg/kg) did not affect the acute hyperactivity produced by MDPV (0.5 - 3 mg/kg). However, nasal administration of NAAG (10, 100 and 500 µg/10 µL) did reduce MDPV-induced hyperactivity, but only at the highest dose tested. Furthermore, both 2-PMPA (1, 10 and 30 mg/kg) and NAAG (10, 100 and 500 µg/10 µL) dose-dependently attenuated MDPV (2 mg/kg)-induced conditioned place preference. The current findings demonstrate that MDPV withdrawal produces dysregulation in the endogenous NAAG-GCPII signaling pathway in corticolimbic circuitry. Targeting this system through systemic administration of the GCPII inhibitor 2-PMPA, or NAAG itself, attenuates MDPV drug-seeking behavior.
Keywords: MDPV, bath salts, glutamate, 2-PMPA, NAAG, GCPII, mGluR2/3, conditioned place preference

Disclosures: C. Hicks: None. **R.A. Gregg:** None. **S.U. Nayak:** None. **C.S. Tallarida:** None. **A.B. Reitz:** None. **G.R. Smith:** None. **S.M. Rawls:** None.

Poster

548. Drug Addiction: Translational Studies

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Program#/Poster#: 548.10/KKK1

Topic: G.08. Drugs of Abuse and Addiction

Support: Canada Research Chairs (CRC 950-229982)

Title: On Target effect of Mu opioid receptor activation identified by mouse rsfMRI and Spectroscopy

Authors: *E. DARCQ[1], M. NASSEEF[1], J. NEAR[1], B. L. KIEFFER[1,2];
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Abstract: In the area of psychiatric disorders, success in developing effective drugs from highly promising molecular targets has been limited. Although drug-induced molecular processes are

well understood at the cellular level, and behavioral effects have been characterized in animal models, the overall impact of target activation and inhibition on neural network structure, neurochemistry and connectivity at the whole-brain level remains largely unknown. Our goal is to develop a new platform to identify and screen novel drugs in the area of psychiatric disorders, using combined resting state functional Magnetic Resonance Imaging (rsfMRI) and Spectroscopy (MRS) in living mice. Here we present a proof-of-principle study based on Mu opioid Receptor (MOR) responses to opiate drugs. MORs are expressed in brain areas belonging to pain and addiction circuits and, using gene knockout (KO) in mice, our laboratory demonstrated that this particular opioid receptor mediates both the remarkably potent analgesic and addictive properties of opiates like morphine [1]. MOR is a prime target in the development of novel potent analgesics, possibly devoid of adverse effects, and also new treatments for substance use and mood disorders [2-3]. Our approach consists in measuring morphine-induced modifications of Blood oxygen level dependent (BOLD) signal in the brain of live wild-type (WT) and MOR KO animals. Images are acquired using 7 Tesla Scanner with morphine injection after five minutes of initial scan. Functional data are preprocessed using standard pipeline. Activation maps are then established, functional connectivity is characterized for key selected regions by subgrouping before and after morphine injection and finally, information flow is characterized within small networks using Granger causality [3] on both group and subject level [4]. Comparing WT and MOR KO data will allow subtracting non-specific effects, and establish the on-target signature of MOR-mediated activation of neural networks in living animals. We also characterize neurotransmitter modifications by 1H-MRS in a MOR-enriched region (habenula) to profile neurochemical modifications. Altogether, the combined characterization of activation, connectivity and neurochemical effects induced by the prototypic opiate drug provides a reference dataset to further test other mu opiates used in the clinic and under development. [1] Matthes et al, Nat. 383(6603),819-23,1996. [2] Lutz et al,Trends Neurosci. 36(3),195-206,2013. [3] Fava et al, Am J Psychiatry. 173(5),499-508,2016. [4] Barnett et al, J Neurosci Methods 223,50-68,2014. [5] Schreiber et al, Physica D 142,346-382,2000

Disclosures: **E. Darcq:** None. **M. Nasseef:** None. **J. Near:** None. **B.L. Kieffer:** None.

Poster

548. Drug Addiction: Translational Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH HHSN275201500005C

California TRDRP Grant 24RT-0023

Title: Selective small-molecule agonists for the nociceptin opioid (NOP) receptor attenuate ethanol-induced conditioned place preference in mice

Authors: *N. T. ZAVERI[1], M. E. MEYER[1], V. B. JOURNIGAN[1], P. V. MARQUEZ[2], A. HAMID[2], K. LUTFY[2];
[1]Astraea Therapeutics, LLC, Mountain View, CA; [2]Col. of Pharm., Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Alcohol-related disorders represent major public health issue and places tremendous burdens on society and economy. However, a very limited number of pharmacotherapies are available to treat alcoholism and related disorders. A growing body of evidence suggests that the nociceptin opioid receptor (NOP, previously known as opioid receptor-like (ORL1) receptor) may be a potential target to treat alcohol addiction. Indeed, nociceptin/orphanin FQ (N/OFQ), the endogenous peptide agonist for the NOP receptor, has been shown to reduce the rewarding action of alcohol in a mouse conditioned place preference (CPP) assay. Further, a small-molecule NOP agonist Ro 64-6198 also reduced the acquisition and reinstatement of alcohol CPP in mice. Here we examined the effect of a series of novel, selective and potent small-molecule NOP receptor agonists on the rewarding action of ethanol using the CPP paradigm as an animal model of ethanol reward. Mice were tested for preconditioning place preference on day 1; in which mice were placed in the central neutral chamber of the CPP apparatus and allowed to explore the chambers for 15 min. The amount of time that mice spent in each chamber was recorded. On days 2-4, mice were treated with vehicle or one of the NOP agonists (AT-312, AT-328, AT-202 and a known NOP agonist SCH221510), followed by saline/ethanol (2 g/kg) or ethanol/saline administration and conditioned to the CPP chambers for 15 min. Mice were then tested under a drug-free state for postconditioning place preference on day 5, as described for day 1. Our results revealed that AT-312 and AT-328 that exhibit high affinity toward the NOP receptor compared to AT-202, abolished ethanol CPP in wild-type mice. In contrast, these compounds failed to alter the CPP response in mice lacking NOP, suggesting that the NOP receptor mediates the inhibitory action of these compounds on alcohol reward. Together, these data suggest that the NOP receptor may be a potential pharmacotherapeutic target to develop medications to treat alcoholism and alcohol-related disorders. (Supported by HHSN275201500005C to NTZ and TRDRP Award 24RT-0023 to KL)

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Poster

548. Drug Addiction: Translational Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA P50 grant DA027840

NIH/NIAAA R03 grant AA022479

Title: Differential effects of ketamine on alcohol intake in male vs female alcohol-preferring (P) rats

Authors: *A. H. REZVANI[1], E. D. LEVIN[2], M. CAULEY[2], B. GETACHEW[3], Y. TIZABI[3]; [1]Dept. of Psychiatry, [2]Duke Univ., Durham, NC; [3]Howard Univ., Washington DC, DC

Abstract: The complex neurobiological substrates of drug addiction in general and alcoholism in particular may be responsible for lack of optimal interventions in these disorders. Treatment strategies for alcoholism include disulfiram, naltrexone and acomprosate, which target the metabolism of alcohol, opioid receptors or variety of other neurotransmitters, respectively. Recently, a major role for the glutamatergic system in alcohol addiction has been suggested. This study was conducted to test the effectiveness of ketamine, an NMDA receptor antagonist on alcohol consumption in P rats. Moreover, since gender differences in response to alcohol and other drugs have been demonstrated, both male and female rats were studied. Adult male and female P rats originally obtained from Indiana University, but bred locally at Duke University were given 24 h access daily to two bottle choice (10% alcohol or water) for a period of over 1 month. These animals were then injected with various doses of ketamine (0, 5, 7.5 and 10 mg/kg s.c.) and their alcohol consumption was recorded at 2, 4, 6 and 24 Hr. A dose-dependent effect of ketamine in both male and female rats was observed, although the total alcohol consumption was lower in vehicle-treated female compared to vehicle-treated male rats. Moreover, it appears that the female rats are much more sensitive to ketamine's effect. Thus, 10 mg/kg ketamine resulted in approximately 26% decrease in alcohol intake in male, but 48% decrease in female alcohol consumption over the 24h period. Ketamine, a potent dissociative anesthetic, has been shown to have rapid and lasting antidepressant effects at doses much below the anesthetic dose, equivalent to the doses used in this study. Ketamine itself may be a drug of abuse, thus its usefulness in treatment of alcoholism may not be a viable option. However, identification of specific NMDA or other glutamate receptor subtypes that may be responsible for reducing alcohol consumption could hold the key to novel intervention in alcoholism or alcohol use disorders. The effectiveness of the drug for inhibiting nicotine self-administration is also being tested. Supported by NIH/NIDA P50 grant DA027840 and NIH/NIAAA R03 grant AA022479.

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Poster

548. Drug Addiction: Translational Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH NIAAA grant U01AA023489

Title: Fyn kinase in the dorsomedial striatum controls action-outcome association to drive goal-directed alcohol responding

Authors: ***N. MORISOT**[1], K. PHAMLUONG[1], A. CROSS[2], D. RON[1];
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Abstract: Fyn is a non-receptor tyrosine kinase, crucial for synaptic function (1). We previously found that excessive alcohol drinking in rodents promotes Fyn activation in the dorsomedial striatum (DMS), resulting in Fyn-mediated phosphorylation of GluN2B which enhances NMDARs activity (2, 3). Importantly, we found that Fyn signaling in the DMS contributes to alcohol-drinking behaviors (2-6). AZD0530 is an oral Fyn inhibitor developed for the treatment of Alzheimer's disease and cancer (7, 8). We therefore tested the potential use of AZD0530 in the treatment of alcohol use disorders (AUDs). First, we confirmed that systemic administration of AZD0530 prevents alcohol-induced Fyn activation in the DMS of mice. Importantly, we showed that systemic administration of AZD0530 in mice decreased alcohol intake and seeking in an operant self-administration paradigm. We further demonstrated that intra-DMS infusion of AZD0530 reduced alcohol self-administration and seeking in rats. Given that the DMS controls goal-directed behaviors, which is a key step in the development of AUD (9), we hypothesized that Fyn in the DMS drives goal-directed alcohol use. Goal-directed actions rely on the association between the action and the outcome and therefore are sensitive to changes in i) the outcome value and ii) action-outcome contingency. To test whether Fyn in the DMS contributes to processing changes in alcohol value to drive goal-directed alcohol seeking, we used a devaluation test. We found that systemic or intra-DMS administration of AZD0530 prior to alcohol devaluation did not affect the devaluation sensitivity in mice and rats, revealing that Fyn in the DMS does not signal alcohol value to promote goal-directed alcohol seeking. We then tested whether Fyn is important for the contingent association between lever pressing and alcohol delivery, using a contingency degradation test. We found that systemic administration of

AZD0530 blocks the sensitivity to contingency degradation in mice, revealing a role for Fyn in underlying the contingency between lever-pressing and alcohol delivery that drives goal-directed alcohol behaviors. Together, our results suggest that AZD0530 may be used for the treatment of AUDs. Our findings further reveal a critical role for Fyn in DMS-dependent mechanisms that drive goal-directed alcohol intake and seeking.

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Poster

548. Drug Addiction: Translational Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: National Institute on Drug Abuse Intramural Funding

Title: Characterizing the structure-function relationships of atypical dopamine uptake inhibitors with fast scan cyclic voltammetry and microdialysis i mice

Authors: *J. KEIGHRON[1], A. H. NEWMAN[2], G. TANDA[2];
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Abstract: Atypical dopamine uptake inhibitors (DUIs) such as Modafinil and compounds derived from it are potent potential treatments for psychostimulant abuse disorders, and the relationship between each of these compounds and the result effect on the dopamine transporter (DAT) are of vital interest for the development of treatments. In the past two decades several advances into understanding how the DAT is affected by both typical (cocaine, etc.) and atypical (Modafinil, JHW 007, etc.) DUIs have been published. Here, we demonstrate how the structural changes in DAT caused by DUI binding relate to the dopamine (DA) actions of DAT. By studying several known DUIs (cocaine, R-Modafinil, JHW 007, WIN 35 428) and structurally related derivative compounds (JJC8-016, JJC8-088, JJC8-091) with both microdialysis (MD) and Fast Scan Cyclic Voltammetry (FSCV) in male Swiss-Webster mice we have been able to draw direct correlations to binding studies with wild type and mutant (Y335A, Y156F) DAT in vitro. The data indicate that (1) blockade of DA clearance by DAT is strongly correlated with the DAT affinity for each compound. That (2) the duration of effect for each inhibitor tested correlates well with the DAT conformation as demonstrated by the Y335A/WT binding ratios with both techniques, which indicates that DUIs with a smaller ratio of Y335A/WT binding are more likely to be in an inward or occluded conformation. Finally that (3) the interactions of atypical

inhibitors with Tyr156 may play a role in the tonic (MD) and phasic (FSCV) concentrations of DA, as demonstrated by the correlation between Y156F/WT binding ratio and DA concentrations. Together, these results provide insight into the design of future treatments for psychostimulant abuse disorders by elucidating the relationships behind the DAT structure with DUIs bound and its function.

Disclosures: J. Keighron: None. **A.H. Newman:** None. **G. Tanda:** None.

Poster

548. Drug Addiction: Translational Studies

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Program#/Poster#: 548.15/KKK6

Topic: G.08. Drugs of Abuse and Addiction

Support: Canada Research Chairs (CRC) 950-229982

Title: Rs-fMRI directional connectivity is modified in conditional mice lacking the mu opioid receptor gene in striatal neurons

Authors: *M. T. NASSEEF[1], E. DARCQ[1], A. MECHLING[2], D. V. EVERFELDT[2], J. HENNIG[2], L.-A. HARSAN[2,3,4], B. L. KIEFFER[1,5];
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Abstract: In humans, resting state magnetic resonance neuroimaging (rsfMRI) has opened the era of connectome/imaging genetics, in order to elucidate how genetic factors affect brain organization and functional connectivity in healthy individuals and disease. Yet the causal impact of a single gene on functional and directional information flow remains largely unknown, and animal research is best suited to this goal. Here, we tested whether functional MRI in living animals would reveal connectivity alterations upon total or conditional targeted inactivation of a single gene, the mu opioid receptor (MOR) gene. MOR is broadly distributed throughout the nervous system and mediates the remarkably potent analgesic and addictive properties of opiates like morphine. This receptor facilitates rewarding effects of both drugs of abuse [1] and social interactions [2] with potential implications for autism [3]. Here we explore the influence of striatal MORs on whole-brain connectivity in live animals by rs-fMRI. We created conditional

knockout(cKO) mice, using targeted inactivation of the MOR gene in GABAergic neurons of the forebrain (Charbogne et al, submitted) and obtained mutant mice with complete MOR deletion specifically in the striatum, a brain area essential for reward and motivation processing. Functional rsMRI images were acquired on a 7T Bruker scanner as previously described[4], and preprocessing performed using a homemade template and a standard pipeline. Voxelwise functional connectivity(FC) maps with group difference and Granger Causality (GC) [5], a measure of directed causation, were performed using standard seed analysis. Subject level analysis was implemented by generating Iterative Multivariate Amplitude Adjustment Fourier Transformation surrogates(IMAAFTs)[6]. Our data show pronounced modifications of voxelwise FC maps in cKO mutant animals, and group differences reveal significant changes in several regions of the brain, in particular striatum, habenula and VTA. Further, GC analysis of selected small networks shows distinct dominant directionality in cKO mutant mice versus controls, following both population and single subject IMAAFTs analysis. Our work demonstrates that MOR activity in the striatum regulates brain activity under resting state, which is detectable by rsfMRI in live animals, and is characterized by intrinsic functional and directional information flow signatures.

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[1] Contet et al, Curr. Opin. Neurobiol,2004
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[4] Mechling et al, Neuroimage,2014
[5] Barnett et al, J Neurosci Methods,2014
[6] Schreiber et al, Physica D,2000

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Poster

548. Drug Addiction: Translational Studies

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Program#/Poster#: 548.16/KKK7

Topic: G.08. Drugs of Abuse and Addiction

Title: Aberrant resting state functional connectivity of the insula in internet gaming disorder

Authors: *K. KIM[1,2], D. LEE[1,2], J. LEE[1,2], Y. JUNG[2];
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Abstract: Objectives : Internet gaming disorder (IGD) is defined as the excessive and uncontrolled internet gaming behavior despite a variety of negative psychosocial consequences. Previous studies have reported that many aspects of IGD are similar to those of substance addiction. The insula serves as a key neural substrate in substance addiction, playing a crucial role in salience processing and interoceptive awareness. The insula is subdivided into distinct subregions that have different connections and functions. The anterior insula is embedded in the salience network, which facilitates access to attention by switching between large-scale networks when a salient event is detected. The posterior insula is functionally involved in interoception and sensorimotor information. The aim of this study is to evaluate the insula subregions based network in young adults with IGD, using the resting-state functional magnetic resonance imaging (fMRI). **Methods :** The participants were 14 young male adults with IGD (mean age=23.6±2.6) and 20 age-matched male healthy controls (HCs). Resting state functional magnetic resonance image data were obtained from subjects during a 6 minute passive-viewing block scan. We selected the right and left insula subregions (anterior, posterior) as seed regions in a connectivity analysis. **Results :** In comparison with the HC group, the IGD group demonstrated decreased functional connectivity between the right anterior insula and dorsal attention network related regions including frontal eye field (FEF) and superior parietal lobule (SPL). The IGD group also show significantly stronger functional connectivity between the right posterior insula and subgenual anterior cingulate cortex (sgACC). **Conclusion :** Our results suggest that young male adults with IGD exhibit aberrant insula-based network. Participants with IGD demonstrated weaker insula-centered resting state functional connectivity with brain regions implicated in attentional processes. They also showed abnormal connection between the insula and sgACC which has been related to craving in addiction. These resting-state functional connectivity findings might underpin a biological basis for IGD and are functionally related to disruption of salience processing and interoceptive awareness in young adults with IGD.

Disclosures: K. Kim: None. **D. Lee:** None. **J. Lee:** None. **Y. Jung:** None.

Poster

548. Drug Addiction: Translational Studies

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Program#/Poster#: 548.17/KKK8

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA R21DA035461

Title: Effect of Vasopressin and Oxytocin on Adrenocorticotropic hormone in cocaine dependent patients

Authors: M. S. HELLER[1,2], *W. N. RABY[1,2];
[1]Columbia Med. Ctr. Dept. of Psychiatry, New York City, NY; [2]Dept. of Psychiatry, Albert Einstein Col. of Med., Bronx, NY

Abstract: Aims: Chronic stress models describe Vasopressin (VP) control over ACTH secretion and reduced regulatory control by Oxytocin (OT) as consequences of stressor chronicity. If cocaine-dependence is a form of chronic stress, do these mechanisms apply? In cocaine-dependent patients (CDP) we measured serum ACTH levels under 3 conditions: 1) after administration of intranasal (IN) Desmopressin (DDAVP); 2) in response to IN DDAVP after a pretreatment with IN OT; 3) after 6-wks of IN OT vs. placebo (PBO). This study is conducted as part of a 6-wk clinical trial investigating if daily IN OT (24 IU) can reduce relapse risk in CDP.
Methods: Phase 1 tests effects of IN DDAVP vs. IN OT on serum ACTH in consented CDP (n=18) relative to healthy controls (CON;n=8) after abstinence is reached. Testing begins with a 2-hr habituation period. On Day 1, ACTH is collected at 30',20',10' prior to 80 IU DDAVP and 10',20',30'45',90' after. On Day 2, ACTH is collected at 30',20',10' before 20 IU IN OT, at 10',20',30' after; and 10',20',30',45',90' after 80 IU IN DDAVP. Phase 2 is a 6-wk, double-blind, randomized, PBO-controlled trial of daily IN OT vs. PBO for CDP. Phase 3 repeats Day 1 of Phase 1.
Results: Compared to baseline, ACTH levels increase in CDP (t=4.65;p=0.0002) and CON (t=3.62;p=0.0086) after IN DDAVP. However, ACTH levels are not elevated in both CDP (t=0.951;p=0.3547) and CON (t=0.909;p=0.3941) after IN OT. In CDP, compared to the effect of IN DDAVP alone, pretreatment with IN OT at study entry reduced ACTH secretion induced by IN DDAVP (t=4.65;p=0.0002). This effect was not seen in CON (t=0.295;p=0.7802). To date for 8 CDP who completed 6-wks of IN OT vs. PBO, the effect of IN DDAVP on ACTH did not differ from study entry (t=1.05;p=0.3329).
Conclusions: IN DDAVP elevates ACTH in CDP and CON, IN OT by itself does not. Pretreatment with IN OT appears to block the ACTH-stimulating effect of IN DDAVP in CDP, but not in CON. So far, 6 weeks of treatment with IN OT or PBO does not alter the ACTH response to IN DDAVP in CDP. Considering that stress mechanisms have repeatedly been observed to perpetuate cocaine addiction, the sensitivity of cocaine-dependent patients to exogenous OT opens a possible treatment approach, based on reinstating regulatory control over CNS-based stress mechanisms. The impact on relapse risk in cocaine-dependent patients is presently being investigated in the clinical trial phase of this study.

Disclosures: M.S. Heller: None. **W.N. Raby:** None.

Poster

548. Drug Addiction: Translational Studies

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Topic: G.08. Drugs of Abuse and Addiction

Support: DA033533 (N.S)

DA037294 (N.S.)

AA022082 (F.W.)

DA039821 (F.W.)

Title: Transdermal CBD attenuates cocaine intake in rats with addiction-linked cocaine history

Authors: F. WEISS[1], *A. LAQUE[2], G. WAGNER[1], G. DENESS[1], T. KERR[1], D. WATRY[1], N. SUTO[1];
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Abstract: A major challenge for successful treatment of cocaine addiction is to effectively reduce both chronic cocaine intake and long-lasting susceptibility to relapse. Various medications to treat cocaine addiction are currently in use, but with largely unimpressive success rates. Previously our lab identified possibly substantial potential of cannabidiol (CBD) - a major non-psychoactive component of *cannabis sativa* - for cocaine relapse prevention. Where treatment with CBD via the transdermal (i.e., a clinically relevant) route reduced both cue- and stress-induced reinstatement of cocaine seeking in rats. Here, we sought to determine whether tCBD's therapeutic effects extend to cocaine self-administration. Wistar rats were divided into two separate cohorts based on their histories of cocaine self-administration: extended history (12wk; serving as a rat model of cocaine addiction) and short history (2wks). All rats were treated at 24h intervals with tCBD (0, 15, 30 mg/kg) for nine days. Cocaine self-administration tests were conducted on treatment days 3-9 under a FR1 schedule of reinforcement. In these experiments, both doses of tCBD reduced cocaine self-administration in rats with an extended cocaine history. Moreover, tCBD produced these actions without non-specific behavioral suppression or tolerance to the drug's "therapeutic" effects with repeated treatment. However, neither tCBD dose was effective at reducing cocaine intake in rats with a short cocaine history. These observations suggest that tCBD may reverse neuroadaptive changes underlying cocaine motivation linked to histories of long-term chronic cocaine use - rather than to 'recreational' or brief exposure. Thus, clinically relevant transdermal application of CBD may have unique potential for the treatment of cocaine addiction at the level of both drug intake and relapse.

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Poster

548. Drug Addiction: Translational Studies

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Title: Non-invasive brain stimulation for addiction: can we boost a hypothetical frontal ephaptic signaling of theta/gamma waves?

Authors: *J. F. GOMEZ-MOLINA[1], U. M. RICOY[3], M. CORREDOR[4], L. F. BOTERO-POSADA[5], J. VELEZ[2];
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Abstract: INTRODUCTION 1. TMS activation of some regions of the PreFrontal Cortex (PFC, like DLPFC and DMPFC) cause a therapeutic effect on addiction (Dunlop et al 2016) 2. Frontal theta is a signal of cognitive control (Cavanagh et al 2014) and it is modulated by alcohol intoxication (Marinkovic et al 2012) 3. Electric fields precede electrochemical communication and guides network activity (Frohlich and McCormick 2010) 4. Electric fields are fast enough and far reaching enough to functionally organize the brain. They generate an activity that appears as "self-controlled" (Llinas, 2001) 5. Important ephaptic effects can be caused by waves and traveling depolarizations (Gomez-Molina, Restrepo Velazquez, Botero-Posada 2015 http://repository.eia.edu.co/revistas/index.php/BME/article/view/732). HYPOTHESIS. Population bursts, traveling along aligned cell membranes in frontal lobes (pyramidal dendrites and axons), represent an ephaptic signal for cognitive control of addictive behaviors. Non-invasive brain stimulation (NIBS), when applied at Fp1 or F3 (EEG-coordinates), can amplify the wave-form of this bursting causing 2 therapeutic effects: 1. It can increase the strength of frontolimbic connections by Ca2+-dependent plasticity mechanisms (Gomez, Ricoy, Escobar, Velez, SfN-abstract 2013) 2. It can cause important ephaptic effects that can guide network activity. PROPOSED METHODS 1. Electrophysiological and Engineering analysis and design

2. EEG/MEG-inverse solutions to determine the intracranial electric fields 3. Cognitive methods/therapies or meditation techniques designed to enhance frontal cognitive control can be simultaneous to NIBS. DISCUSSION 1. The existence of this effects is suggested based only in theoretical considerations and indirect evidence 2. The development of sensitivity to weak fields gives important advantages and exotic properties to brain cells that conventional methods of communication (synaptic, diffusive, conventional spiking etc) does not have 3. Ideally, friendly NIBS should work by mimicking or amplifying neural correlates of frontal will action. CONCLUSIONS 1. NIBS (e.g. Theta burst stimulation) can boost possible ephaptic effects generated by will action. 2. Although generally considered too weak to have a major role in controlling brain activity, electric fields have the potential of produce non-local, global effects that can be rapidly amplified to change networks and functional connectivity in certain phases. 3. Gamma-theta activity can represent a hypothetical correlate of a "self-determined", free will mechanism susceptible of addiction therapy.

Disclosures: J.F. Gomez-Molina: None. **U.M. Ricoy:** None. **M. Corredor:** None. **L.F. Botero-Posada:** None. **J. Velez:** None.

Poster

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NIDA 2P50DA18165

This material is the result of work supported with resources and the use of facilities at the VA Portland HCS

Title: Parametric BOLD activation by a discounting task predicts sobriety at three months in alcohol dependent subjects

Authors: *W. F. HOFFMAN[1,2], L. DENNIS[2], H. MCCREADY[2], D. SCHWARTZ[3], B. TREMBLAY[3], M. KOHNO[3];
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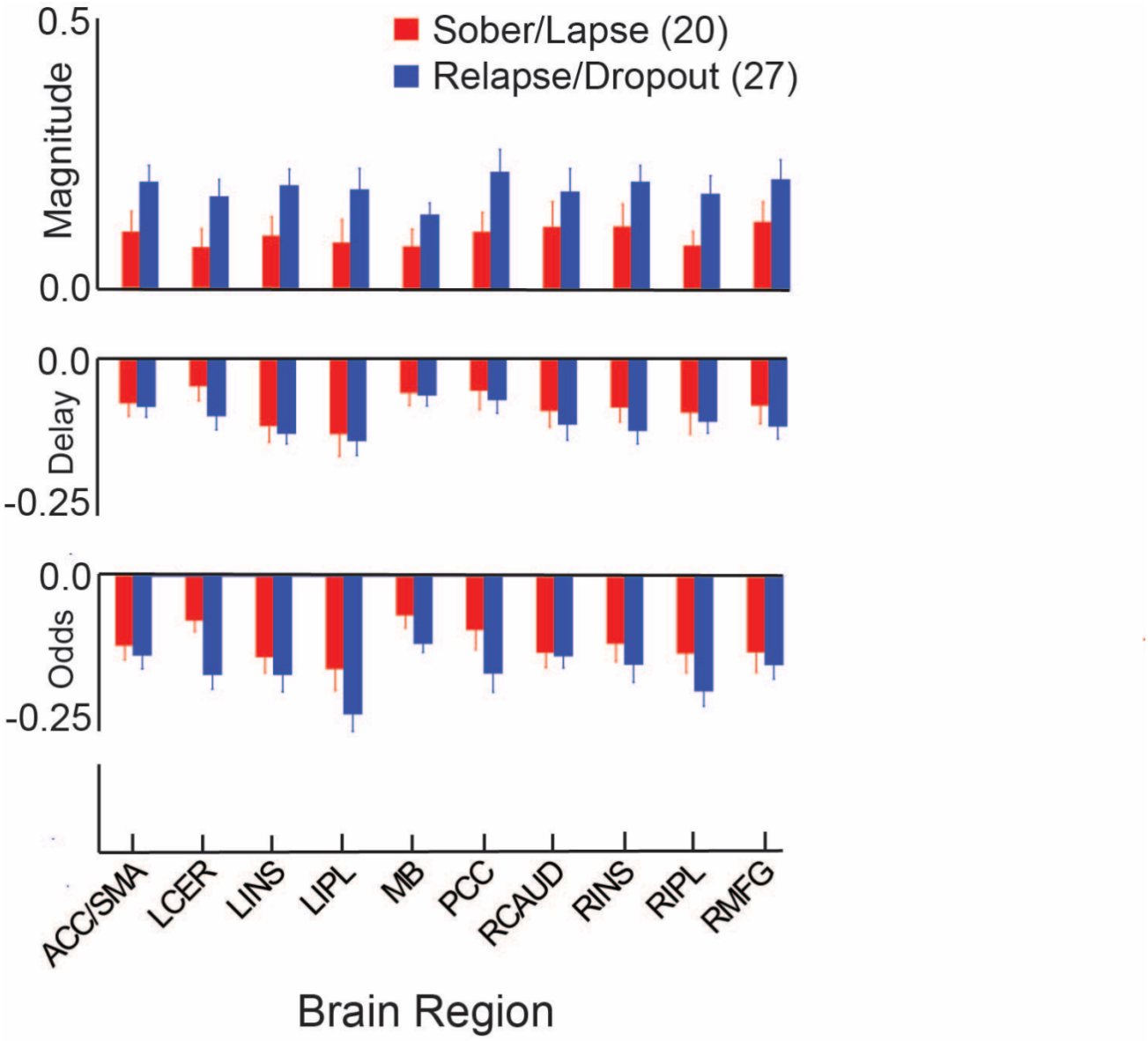
Abstract: Subjects: Forty-seven subjects who met DSM-IV TR criteria for alcohol dependence were enrolled in a prospective study to test the hypothesis that neural phenotypes associated with probability and delay discounting could predict sobriety at three months. Subjects averaged 19 ± 11 standard drinks per day, had, at minimum, problem drinking for 3 years and were 2-4 weeks abstinent at study entry.
Methods: Subjects were evaluated with an fMRI Probability Delay Discounting task. For each choice, the subject was asked to choose between a fixed, immediate, 100% certain reward of $20 and an alternative reward. The alternative varied by odds against ([1-p]/p) from 0 to 3 (100% to 25% likelihood of success), delay from 0 to 12 months and monetary reward amount from $20 to $140.
A drinking diary was reviewed at each of three monthly follow-up visits. Twenty subjects had minor lapses (non-heavy drinking on no more than 2 consecutive days) or remained sober, while 27 subjects relapsed (one or more days of heavy [more than 5 drinks/day] drinking) or were lost to follow up and presumed relapsed.
fMRI Analysis: fMRI scans were preprocessed in a standard manner with AFNI. We identified brain regions sensitive to parametric variation of reward magnitude, delay and odds against with amplitude modulated regression. We chose a set of brain regions where activation by the three cardinal characteristics overlapped and met the cluster size criterion of 200 voxels at a voxelwise p = .001. We created a mask from these regions and extracted average regional values of the parametric regressors for magnitude, delay and odds against. **3 Month Outcome:** We used a linear mixed effects model to test the hypothesis that the value of the parametric regressors at baseline predicted outcome at three months.
Results: Subjects who relapsed or dropped out were more sensitive to the magnitude of reward (p = .046), delay (p = .095) and odds against (p = .082) (**Figure).**
Conclusion: Neural response to characteristics of discounted rewards may be a useful predictor of treatment outcome in recovering alcohol dependent individuals.



Mean regional parametric regressor for Magnitude, Delay and Probability (Odds against) predicts outcome three months after initial sobriety. Linear mixed effects model of outcome: Magnitude, p = .046, Delay, p= .095, Odds Against, p = .082. ACC/SMA: anterior cigulate cortex/supplementary motor area; LCER: left cerebellum; LINS: Left insula; LIPL: left inferior parietal lobule; MB: midbrain; PCC: posterior cingulate cortex; RCAUD: right caudate; RINS: right insula; RIPL: right inferior parietal lobule; RMFG: right middle frontal gyrus. Error bars are SEM.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIAAA RO3AA023268

Title: Effects of Acamprosate, Naltrexone and $CaCl_2$ on cognitive flexibility and synaptic function in mice following chronic-intermittent ethanol (CIE) exposure or operant alcohol self-administration

Authors: *A. J. PHENSY[1], G. PRADHAN[2], R. KANDUNURI[2], M. RAZZAQUE[2], M. PARKER[2], A. CARRASCO[2], S. KROENER[2];
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Abstract: Alcohol abuse leads to functional impairment of the prefrontal cortex (PFC), which weakens inhibitory control over excessive drinking, leading to increased alcohol consumption, forming a vicious cycle that contributes to alcohol addiction and dependence. Acamprosate is the leading medication approved for the maintenance of abstinence, shown to reduce craving in both animal models and human alcoholics. Acamprosate may reduce relapse by restoring balance of glutamatergic synaptic transmission in the PFC. We have recently shown that (passive) CIE exposure enhances N-methyl-D-aspartate receptor (NMDAR) function, causing aberrant synaptic plasticity in the PFC and disruption of normal PFC function. CIE exposure led to decreased performance in an attentional set shifting task as well as an increase in synaptic NMDA currents at PFC pyramidal neurons (Kroener et al., 2012). Treatment with acamprosate prior to testing recovered the behavioral deficit but did not significantly affect the synaptic dysfunction (Hu et al., 2015). In order to further investigate how acamprosate affects cognitive flexibility and alcohol intake, we compared the effects of 2 doses of acamprosate (100 and 200 mg/kg) to the effects of CaCl2 (the active moiety of acamprosate) and the opioid antagonist naltrexone in our attentional set-shifting task, as well as a novel object recognition and social recognition task following CIE. Animals treated with either acamprosate, naltrexone or CaCl2 for three days following 3 cycles of CIE showed a reversal of the alcohol-induced deficits in cognitive flexibility in the attentional set shifting task. Novel object recognition and social recognition were unaffected by CIE. In order to better distinguish alcohol-induced effects on learning and goal-directed behavior from the pharmacological effects of high-level alcohol exposures (as they occur in CIE), we also examined if mice that self-administer alcohol in brief (30 min) daily training sessions show similar deficits in behavior and synaptic plasticity, and if so, whether these changes can be recovered by acamprosate. Following operant conditioning, animals were tested for behavioral deficits and then sacrificed to investigate synaptic changes through whole

cell patch clamp recordings. Animals showed impairment in the attentional set shifting, as well as an upregulation of the NMDA:AMPA ratio at PFC pyramidal neurons. Acamprosate ameliorated the behavioral deficits and recovered NMDA:AMPA ratios similar to those of water-drinking controls. These experiments will further our understanding of the mechanisms responsible for the transition from controlled drug taking to alcohol addiction.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: The National Research Foundation of Korea 2014M3C7A1062894

Title: Neuronal synchrony in Internet gaming disorder and alcohol use disorder: a resting-state EEG coherence study

Authors: ***J. LEE**[1,2], S. PARK[1,3], M. PARK[1], Y. KIM[1], D. KIM[4], J.-S. CHOI[1,5];
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Abstract: Internet gaming disorder (IGD) is defined as the repetitive use of Internet-based games leading to significant difficulties with social and psychological functioning. Previous studies have reported that patients with addiction problems showed dysfunctional cortical connectivity as well as altered brain functional connectivity. However, there were little evidences about brain connectivity using electroencephalographic (EEG) in patients with IGD. This study aimed to find a biological marker of IGD using resting-state EEG coherence. We analyzed data from a total of 92 male participants (intelligence quotient (IQ) $\geq$ 80), comparing the EEG coherences of 31 patients with IGD who aged 23.39 $\pm$ 5.03, with those of 29 patients with alcohol use disorder (AUD) who aged 30.07 $\pm$ 7.17 and 32 healthy controls who aged 24.97 $\pm$ 3.70 (HCs). The results showed that increased beta (12-25 Hz) coherences in the inter-hemisphere (FP2-T3 pair and F4-T3 pair), midline-left hemisphere (T3-Cz pair) and midline-

right hemisphere (C4-Cz pair and F4-Fz pair) were found in the IGD group compared with the HCs ($P < 0.05$), whereas increased beta coherence in midline-right hemisphere (F4-Cz pair) was found in the AUD group compared with the HCs ($P < 0.05$). It was also figured out that theta (4-8 Hz) and delta (1-4 Hz) coherences were increased in the AUD group compared with HCs in the right frontotemporal hemisphere (theta = FP2-T4 pair and F8-T4 pair; delta = F8-T4 pair; $P < 0.05$). We further analyzed to identify correlation with the severity of IGD and AUD in each patient group. Increased beta coherence was not correlated with the severity of IGD measured by Young's Internet Addiction Test (IAT) in the IGD group. In addition, increased beta, theta and delta coherences in the AUD group were not associated with Alcohol Use Disorder Identification Test (AUDIT) score that indicates the severity of AUD. In conclusion, the result implies that hyperphasic synchrony measured by EEG coherence during the resting state may be utilized for a bio-marker of IGD and AUD. Especially, the different aspects of EEG coherence suggest that it may serve as a neurophysiological marker to discriminate AUD and IGD features.

Disclosures: **J. Lee:** None. **S. Park:** None. **M. Park:** None. **Y. Kim:** None. **D. Kim:** None. **J. Choi:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH NCI CA-113710

Title: Greater frequency of alcohol consumption is associated with a decrease in daily caffeine consumption among cigarette smokers but not non-smokers

Authors: *N. R. GUBNER, N. L. BENOWITZ;
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Abstract: Higher caffeine consumption has been reported among cigarette smokers, which is thought to be in part due to an increase in rate of caffeine metabolism (induction of CYP1A2 enzyme activity) by combustion products in tobacco smoke. In contrast, there is evidence that alcohol may inhibit CYP1A2 activity. Since there are known differences between African Americans and Non-Hispanic whites for alcohol consumption, cigarette smoking, and rate of nicotine metabolism, the current study examined the relationship between caffeine consumption and frequency of alcohol intake in cigarette smokers and non-smokers of both races using data from the National Health and Nutrition Examination Survey (NHANES). A total of 2425 current

smokers, and 7964 non-smokers were identified by combining three waves of the NHANES data from 2007-2012. Participants were divided into 5 groups based on frequency of alcohol consumption (never, <1 day/ week, 1-2 days/ week, 3-6 days/ week, and daily). Caffeine consumption was estimated based on reported dietary intake of caffeine containing beverages. Consistent with previous reports, daily caffeine consumption was higher in Non-Hispanic whites compared to African Americans. Overall, caffeine consumption was higher in smokers versus non-smokers. In African Americans and Non-Hispanic whites there was a significant interaction between alcohol drinking frequency and smoking status for daily caffeine intake (African Americans: $F=13.7$, $p<0.001$, Non-Hispanic whites: $F=19.8$, $p<0.001$). Within both races there was a significant decrease in caffeine consumption associated with greater frequency of alcohol consumption among smokers. In contrast, among non-smokers greater frequency of alcohol consumption was associated with an increase in caffeine consumption. One hypothesis to explain these findings is that alcohol may attenuate the induction of CYP1A2 enzyme activity by cigarette smoking. Smokers who drink alcohol less frequently may have a faster rate of caffeine metabolism that results in greater caffeine intake compared to smokers who drink alcohol more frequently.

Disclosures: N.R. Gubner: None. **N.L. Benowitz:** Other; NLB serves as a paid consultant to pharmaceutical companies that are developing/ market smoking cessation medications and has been a paid expert witness in litigation against tobacco companies.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: P/PROFOCIE-2014-23MSU0140Z-09

Title: Subjects with hazardous alcohol consumption but not alcohol dependence present lower beta mean frequency than subjects with alcohol dependence

Authors: *L. NUÑEZ-JARAMILLO[1], W. V. HERRERA-MORALES[2], L. RAMÍREZ-LUGO[3], J. V. REYES-LÓPEZ[4];
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Abstract: Harmful alcohol use is among the top five risk factors for disease, disability and death worldwide. Hazardous alcohol consumption is a pattern of alcohol consumption that increases the risk of harmful consequences for the user or others, and has been linked with risky behaviors, accidents and injuries. It is noteworthy that hazardous alcohol consumption does not necessarily implies alcohol dependence. While there is a large body of research on the neurophysiological correlates of alcohol dependence there is little information so far about neurophysiological correlates of hazardous alcohol consumption. It has been reported that subjects with hazardous alcohol consumption but not alcohol dependence present increased beta absolute power (AP) at centro-parietal region, and decreased beta mean frequency (MF) in frontal and centro-parietal regions. While increased beta absolute power has been already reported in alcohol dependent subjects, no report has addressed beta MF in these subjects, thus it is not yet known whether this decrease in beta MF is a feature correlated specifically with hazardous alcohol consumption or is also related with alcohol dependence. Herein we performed qEEG analysis of beta MF in subjects with alcohol dependence and in subjects with hazardous alcohol consumption but not alcohol dependence in 19 leads of the international 10/20 system. We found decreased Beta MF in in centro-parietal region in subjects with hazardous alcohol consumption, but not in subjects with alcohol dependence. Our results suggest that differences exist in the neurophysiological correlates of alcohol dependence and hazardous alcohol consumption, supporting the hypothesis that there are differences in the neurophysiological mechanisms underlying these two conditions

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Poster

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Title: A high-fructose diet alters brain insulin signaling, incentive motivation and dopamine reuptake

Authors: *A. R. KOSHELEFF[1], L. TSAN[2], Y. ZHUANG[3], F. GOMEZ-PINILLA[3], S. B. OSTLUND[4], N. P. MURPHY[2], N. T. MAIDMENT[2];

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Abstract: Rewards such as food or drugs increase dopamine (DA) signaling in mesolimbic regions of the brain. With repetition, such increases in DA transmission become associated with proximate cues (e.g., jingles, slogans, paraphernalia), cues that can then trigger further reward-seeking, such as that seen in overeating and drug relapse. Dopamine transporter (DAT) is positively regulated by insulin, such that increasing insulin signaling facilitates DAT activity, thereby promoting DA reuptake and decreasing DA transmission. Conversely, decreasing insulin signaling (e.g., during fasting or due to insulin resistance), downregulates DAT activity. Diets high in sugars and refined carbohydrates impair insulin signaling in the periphery (e.g., Type II Diabetes), and recent evidence suggests insulin resistance can also occur in neuronal tissue. We hypothesize that neuronal insulin resistance due to chronic fructose exposure persistently downregulates DAT activity, leading to reduced extracellular DA clearance, resulting in increased DA signaling that manifests as hypersensitivity to reward-paired cues. Here, we use a commercially available high-fructose pellet chow (66% fructose) known to quickly produce insulin resistance in the periphery. In Exp. 1, we found that, within 3 weeks, this high-fructose diet significantly impaired insulin signaling in the ventral midbrain. In Exp. 2, we compared fructose-exposed with fructose-naïve rats in a Pavlovian-to-instrumental transfer paradigm, a behavioral model of incentive motivation (i.e., cue-induced reward-seeking) for a food reward, where we found increased incentive motivation for a food reward in fructose-exposed rats. In Exp. 3, using in-vivo fast-scan cyclic voltammetry, we found altered electrically-stimulated DA reuptake in the ventral striatum in fructose-exposed rats. These data highlight a potential role for diet and insulin dysfunction in potentiating hypersensitivity to reward-paired cues, an action that may be due to aberrant DAT function.

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Poster

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NIH F32AG047686 to AB

RO1AG044292 to WJ

Title: Enhanced dopamine synthesis capacity in gambling addiction predicts drug-induced increase in reward learning

Authors: *R. COOLS[1,2], R. J. VAN HOLST[1,4], L. K. JANSSEN[1], M. JANSSEN[3], A. BERRY[5], W. JAGUST[6], G. SESCOUSSE[1];
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Abstract: The dopamine hypothesis of gambling addiction is pervasive. Particularly compelling is the clinical observation that patients with Parkinson's disease may develop gambling addiction symptoms following the onset of their dopaminergic therapy (Dagher et al 2009). Moreover, pathological gamblers respond abnormally to dopaminergic drugs (Boileau et al. 2014; & Janssen et al 2015; Zack & Poulos 2007). However there is little to no direct evidence for a categorical difference between pathological gamblers and controls in terms of dopamine transmission at baseline (in a drug-free state). In fact, multiple attempts to provide evidence for the hypothesis that gambling addiction, as in the case of drug addiction (Lee 2009, Martinez 2005, Volkow et al 2011), is accompanied by lower D2 receptor availability than controls have failed (Boileau et al 2014, Clark et al 2012, Joutsa et al 2012, Linnet et al 2010). Here we provide direct evidence for the dopamine hypothesis of gambling addiction by focusing on a different aspect of dopamine transmission. Specifically, we assessed dopamine synthesis capacity with PET 6-[^{18}F]fluoro-L-DOPA (FDOPA) dynamic scans in 15 controls and 15 pathological gamblers. Moreover, we investigated whether differences in dopamine synthesis capacity between gamblers and controls can account for their differential cognitive response to dopaminergic drug administration (Janssen et al 2014). Dopamine synthesis capacity in the striatum was enhanced in pathological gamblers compared with controls. Furthermore, this enhanced dopamine synthesis capacity predicted the effect of the D2 receptor antagonist sulpiride (400mg oral) on reward (versus punishment) learning. Across both gamblers and controls, individuals with high dopamine synthesis capacity exhibited drug-induced enhancement of reward learning, while individuals with low synthesis capacity exhibited attenuation of reward learning. These data provide strong empirical evidence for the pervasive but hitherto unsupported dopamine hypothesis of gambling addiction. They also reinforce the baseline-dependency principle of dopaminergic drug effects, thus demonstrating the importance of a dimensional as opposed to a categorical approach to dopaminergic treatment in psychiatry.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA017949

NIH Grant DA037421

Title: Transgenerational effects of paternal nicotine exposure on fear response and cholinergic function

Authors: ***M. G. KUTLU**, R. COLE, J. M. TUMOLO, V. PARIKH, T. J. GOULD; Temple Univ., Philadelphia, PA

Abstract: Tobacco use is the leading cause of preventable death in the US as it directly leads to various negative health consequences including cardiovascular diseases and cancer. In addition, numerous studies have indicated a relationship between smoking and mental health problems such as anxiety and stress disorders. Recent developments in genetics have revealed that the effects of exposure to addictive drugs such as cocaine and nicotine are not confined within the same generation but transgenerationally transmitted through epigenetics mechanisms. However, effects of paternal nicotine exposure on pathogenesis of fear and anxiety-related disorders in subsequent generations are virtually unknown. Therefore, in order to investigate the transgenerational effects of paternal nicotine exposure on fear learning and anxiety, we conducted a series of experiments where male adult C57BL6/J mice received either chronic nicotine (28 days, 12.6 mg/kg/d) or chronic saline exposure. The offspring of nicotine (Nic-Sired) and saline (Sal-Sired) exposed mice were tested in contextual and cued fear conditioning. Our results demonstrated that paternal nicotine exposure resulted in augmented cued and contextual fear learning in the subsequent (F1) generation compared to Sal-Sired mice. This effect was reversed when F1 generation mice received acute nicotine injections. The paternal nicotine exposure-induced augmentation of fear learning also persisted in the F2 generation. Moreover, Nic-Sired mice also showed more pronounced spontaneous recovery of fear when re-tested following extinction. Importantly, Nic-Sired mice exhibited normal memory function in the non-affective Novel Object Recognition paradigm, suggesting the effect of paternal nicotine is specific to fear learning. In additional experiments, we did not find any effect of paternal

nicotine exposure in the elevated plus maze or open field tasks, which excluded the possible confounding effects of anxiety and locomotor activity. Finally, using an electrochemical recording technique, we also measured potassium and nicotine-evoked acetylcholine release in the dorsal and ventral hippocampus. Our results showed reduced ventral, but not dorsal, hippocampal cholinergic function in the Nic-Sired mice. In parallel, we also found increased DNA methylation in the ventral hippocampi of the Nic-Sired mice whereas this effects was absent in the dorsal hippocampus. Together, our results suggest that paternal nicotine exposure may result in alterations in the epigenome, which, in turn, leads to exaggerated fear learning and abnormal cholinergic function in subsequent generations.

Disclosures: **M.G. Kutlu:** None. **R. Cole:** None. **J.M. Tumolo:** None. **V. Parikh:** None. **T.J. Gould:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NSFC81171488

Title: Aberrant cerebral functional and structural characteristics of methamphetamine abuser

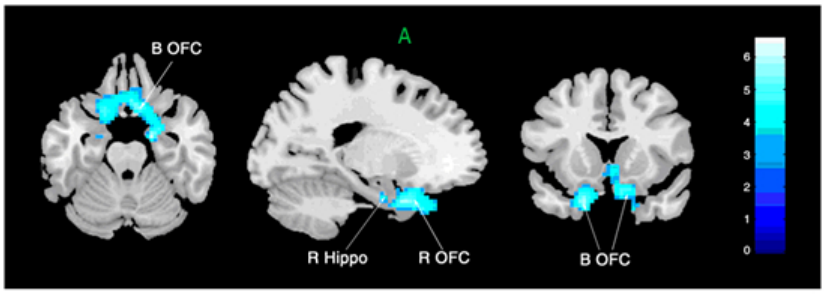
Authors: L. LU, M. ZHOU, L. ZHANG, J. LIU, X. HU, *X.-Q. HUANG;
West China Hosp. of Sichuan Univ., Sichuan, China

Abstract: Purpose
Methamphetamine (MA) is currently a world-widely used addictive psychostimulant. Clarifying the neural mechanisms underlying this problem with MRI can inform more effective treatment.
Method
50 male subjects (mean age=28.74±7.64) met the diagnosis of substance abuse according to DSM-IV and 32 healthy male controls (mean age=33.84±7.22) were recruited and scanned for structural MRI (sMRI) and resting state fMRI (rs-fMRI). ReHo map and subcortical volume of each subject were calculated with DPARSF and Freesurfer software respectively. Between group comparison of ReHo maps were performed using two sample t-test with FWE-correction. The volume of subcortical structures was analyzed using general linear model (GLM) with age and intracranial volume(ICV) as covariates. Relationship between withdraw time and significant regions of sMRI and rs-fMRI were performed with Pearson correlation.
Result

Compared with healthy controls, MA abusers showed lower ReHo in bilateral orbital frontal cortex and right hippocampus(Figure A). MA abusers also showed decreased volume in the right hippocampus, specially, the volume of right hippocampus positively correlated with the withdrawal time(r=0.328,p=0.02). (Figure B)

Conclusion

In present study, we confirm the specific role of hippocampus in MA abusers shown as deficit in regional functional connectivity and volume decrease in the right hippocampus, This is to our knowledge the first study using the ReHo to explore the regional connectivity changes in MA abusers, in additon, we combine sMRI and fMRI to demonstrate the specific role of hippocampus in MA abusers,which had been detected in animal studies before.



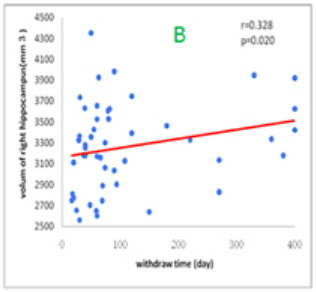


Figure A. Regions showed lower ReHo in MA abusers. B OFC , bilateral orbital frontal cortex (right x=18, y=-3, z=-27, t=6.58, P=0.002;left x=-18, y=15, z=-30 t=5.76,p=0.001) ; R Hippo, right hippocampus (x=6, y=21, z=-18, t=5.89 , P=0.001) .

Figure B. Correlation between volume of right hippocampus and withdraw time.

Disclosures: **L. Lu:** None. **M. Zhou:** None. **L. Zhang:** None. **J. Liu:** None. **X. Hu:** None. **X. Huang:** None.

Poster

548. Drug Addiction: Translational Studies

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 548.29/KKK20

Topic: G.08. Drugs of Abuse and Addiction

Title: Feedback processing during risky decision making in cocaine relapse

Authors: *I. VERVEER[1], R. MARHE[2], D. REMMERSWAAL[1], F. M. VAN DER VEEN[1], I. H. A. FRANKEN[1];

[1]Fac. of social sciences, Erasmus Univ. Rotterdam, Rotterdam, Netherlands; [2]Child and Adolescent Psychiatry, VUmc, Amsterdam, Netherlands

Abstract: Background
Approximately two thirds of substance dependent individuals relapse within months after initiating treatment. This estimation suggests that current treatment interventions in addiction are of limited efficacy. In order to develop more effective interventions, it is important to understand the psychological and biological processes underlying relapse.
Neurobiological substrates that are related to cognitive control problems seem to play an important role in the aetiology and maintenance of substance use disorders (SUD). Therefore, neurocognitive measures of cognitive control functioning in addiction (i.e. event related potentials) may be helpful in predicting relapse and in developing more effective interventions. The current study explored whether the feedback related negativity (FRN) and P3 during risky decision making could predict relapse in cocaine addicted patients.
Method
The FRN and P3 were measured in 21 cocaine dependent patients and 25 healthy controls during the balloon analogue risk taking (BART) task by means of positive (earning money) and negative (balloon pops) feedback. The patient group was tested during their first week of detoxification. Relapse was measured at 3 months follow-up by means of self-reported cocaine use in the last month verified by urine screening (relapse n = 11).
Results
Differences between groups were found in P3 amplitudes related to negative (F (2,43) = 3.930, p = .027) and positive feedback processing (F (2,43) = 3.585, p = .036). Simple contrast analysis revealed reduced P3 amplitudes during negative feedback processing in the relapse group compared to both the non-relapse and the control group (p = .028 and p = .011 respectively). During positive feedback processing, P3 amplitudes were reduced in relapsers compared to the non-relapse group (p = .010). For the FRN, no differences between groups were found. Also, behavioural measures of risk-taking (i.e. number of pumps on the BART task) did not differ between groups.
Conclusions
Reduced feedback related P3 amplitudes measured during the first week of detoxification were associated with relapse at three months follow-up. However, no differences were found in behavioural measures of risk-taking. The results of this study emphasize the importance of neurocognitive measures as possible predictors of relapse and treatment outcome. Future research may therefore focus on treatment interventions modulating neurocognitive measures. Specifically, interventions in addiction that seem to operate by modulating brain activity, like the electrical neurostimulation technique tDCS, are interesting to explore in this respect.

Disclosures: I. Verveer: None. **R. Marhe:** None. **D. Remmerswaal:** None. **F.M. van der Veen:** None. **I.H.A. Franken:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 549.01/KKK21

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant R01DA034806

Title: Taar1 agonists reduce nicotine related behaviors in rats

Authors: *J. LIU[1], J.-X. LI[2];
[1]Pharmacol. and Toxicology, Univ. at Buffalo, SUNY, Buffalo, NY; [2]Dept. of Pharmacol. and Toxicology, SUNY at Buffalo, Buffalo, NY

Abstract: Trace amine-associated receptor 1 (TAAR1), a G protein coupled receptor, modulates the dopaminergic system and dopamine related behaviors. Selective TAAR1 agonists could inhibit the rewarding effects of stimulants, e.g. methamphetamine and cocaine, reducing drug intake and relapse in animal drug addiction model. However, nothing is known about the role of TAAR1 in nicotine addiction or its underlying mechanism. In the present study, we tested the effects of selective TAAR1 full agonist RO5166017 and partial agonist RO5263397 on nicotine related behaviors. First, we tested the effect of both agonists on the maintenance of nicotine taking. Rats were trained to self-administer nicotine (0.003, 0.01, 0.03, and 0.1 mg/kg/infusion) for 1 hr per day under a fixed ratio 3 schedule of reinforcement followed by a 30-s time-out period. Infusions were accompanied by a 5-s illumination of the stimulus light above the active lever and the house light was extinguished for the duration of the time-out period. The results showed that nicotine self-administration followed an inverted U-shaped pattern, with the peak number of injections (about 9-10 injections in 1 hr) maintained by 0.03 mg/kg/infusion. Pretreatment with RO5166017 (5.6 and 10 mg/kg, i.p.) and RO5263397 (3.2 and 5.6 mg/kg, i.p.) 10 min before nicotine self-administration each day dose-dependently decreased the dose-effect curve of nicotine injections and total nicotine intake. We then tested whether activation of TAAR1 would affect the reinstatement of nicotine-seeking behavior. Rats were trained for nicotine self-administration for 15 days in total. The response requirement was gradually increased from FR 1 to FR 3 over a period of 7 days, and then maintained for 8 days under FR 3. Rats were then given six daily extinction sessions, during which lever presses had no consequence (no drug or cues). RO5166017 (10 mg/kg, i.p.) was administrated 10 min before cue- or drug (0.3 mg/kg nicotine, i.p.)-induced reinstatement of nicotine-seeking. Both cue- and drug-induced reinstatement of nicotine-seeking were significantly inhibited by RO5166017. These results indicate that activation of TAAR1 plays an essential role in nicotine-related behaviors, suggesting that TAAR 1 may represent a novel target for the treatment of nicotine addiction.

Disclosures: **J. Liu:** None. **J. Li:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 549.02/KKK22

Topic: G.08. Drugs of Abuse and Addiction

Support: TRDRP Award 24RT-0023

Title: The role of endogenous pituitary adenylyl cyclase activating polypeptide (PACAP) in the motivational effects of nicotine

Authors: ***O. FARHAD**, A. TSENG, P. SINGH, P. MARQUEZ, A. HAMID, K. LUTFY; Pharm., Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Nicotine addiction is a major public health and socioeconomic issue. While the pleasurable effect of low doses of nicotine is thought to play an important role in the initiation of nicotine addiction, the negative affective state that develops following nicotine withdrawal is known to promote relapse. However, the underlying mechanisms of motivational effects of nicotine have not been characterized. The pituitary adenylyl cyclase activating polypeptide (PACAP) and its receptors are widely expressed along the stress and reward circuits, raising the possibility that this system may be involved in the motivational effects of nicotine. The present study sought to determine the role of endogenous PACAP in the aversive and reinforcing effects of nicotine. We used place conditioning and two-bottle choice (TBC) paradigms to assess the role of PACAP in these effects of nicotine. In the TBC paradigm, PACAP knockout and wild-type mice were housed individually while they had access to two water bottles for a week. Mice were then given a choice between water and nicotine (20μg/mL) for the following week. The concentration of nicotine was increased two-fold on each subsequent week. Our results revealed that wild-type mice failed to show any preference for the nicotine solution over the water. On the other hand, mice lacking PACAP consumed more nicotine compared to water. In the place conditioning paradigm, mice lacking PACAP and their wild-type littermates/controls were tested for basal place preference toward the conditioning chambers on day 1. Mice were then injected with either saline or nicotine (1 mg/kg) and confined to either the vehicle-paired chamber or drug-paired chamber, respectively. In the afternoon, animals received the alternate treatment and were confined to the opposite chamber for 15 min. This twice daily conditioning lasted for 8 days. Mice were then tested for postconditioning place preference on day 10. On each test day, mice were placed in the neutral chamber of the CPP apparatus and allowed to explore all the three CPP chambers. The amount of time that mice spent in each chamber was recorded. Our

results showed that wild-type mice spent significantly more time in the saline-paired versus nicotine-paired chamber, showing that wild-type mice exhibited a significant aversion toward the nicotine-paired environment. On the other hand, this aversive effect of nicotine was absent in mice lacking PACAP. Together, these results suggest that endogenous PACAP may mediate the aversive effects of nicotine, and PACAP and its receptors may be a novel target for the development of medications for smoking cessation and nicotine addiction.

Disclosures: O. Farhad: None. **A. Tseng:** None. **P. Singh:** None. **P. Marquez:** None. **A. Hamid:** None. **K. Lutfy:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

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Program#/Poster#: 549.03/KKK23

Topic: G.08. Drugs of Abuse and Addiction

Support: FDA/NIH Grant R01DA037277

Title: Menthol interaction with nicotine to enhance nicotine reinforcement and increase dopamine level in the nucleus accumbens of rats

Authors: Y. GONG, E. HARRISON, L. BISWAS, J. LAGE, *X. LIU;
Pathology, Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Increasing clinical observations suggest that menthol may promote tobacco smoking and nicotine dependence. However, it is not yet clear whether menthol enhances the reinforcing actions of nicotine and interacts with nicotine at neurochemical level. This study employed rat models of nicotine consumption to examine effect of menthol on nicotine self-administration and used intracranial microdialysis to determine influence of menthol on nicotine-induced dopamine release in the nucleus accumbens. Male Sprague-Dawley rats were trained in daily 1-h sessions to press a lever for self-administration of intravenous nicotine at 0.015 mg/kg/infusion, a dose on the ascending limb of the inverted “U” shaped nicotine dose-response curve. Menthol (5 mg/kg) or its vehicle was given intraperitoneally 5 min prior to the test sessions. In separate sets of rats, dopamine levels in the right nucleus accumbens in response to administration of nicotine, menthol, and both were measured using microdialysis coupled with HPLC assay. In the behavioral tests, menthol increased self-administration of nicotine in a dose-dependent manner. In the neurochemical assay, menthol, albeit did not produce an effect on its own, enhanced dopamine release induced by nicotine administration. These data demonstrate a facilitative effect of menthol on nicotine reinforcement and an interaction of menthol with nicotine to enhance

dopamine release in the nucleus accumbens. These findings may shed a light on our understanding of the influence of menthol on smoking and its underlying neurobiological mechanisms.

Disclosures: **Y. Gong:** None. **E. Harrison:** None. **L. Biswas:** None. **J. Lage:** None. **X. Liu:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 549.04/KKK24

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA034389

Title: Interoceptive conditioning and reversal learning with the nicotine and varenicline stimulus

Authors: *B. M. THOMPSON, S. T. BARRETT, J. R. EMORY, C. E. LARSEN, A. M. PEER, S. T. PITTENGER, R. A. BEVINS;
Psychology, Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: Nicotine, the primary psychoactive chemical responsible for the use and addiction to tobacco smoking, has the ability to control behavior. The CDC (2014) estimates that 16.8% of people in the US, roughly 40 million, are smokers. Varenicline (Chantix™) is a smoking cessation drug that works on nicotinic acetylcholine receptors and can reduce the tenacity of the nicotine addiction and facilitates cessation for some smokers. With high relapse rates in individuals who wish to quit, a better understanding of the behavioral and neuropharmacological processes involving nicotine and treatment-related compounds like varenicline will eventually help inform cessation approaches. We have shown that the nicotine stimulus can acquire control over goal-tracking behavior when nicotine predicts intermittent access to brief sucrose rewards. Questions of whether appetitive learning can be reversed or whether varenicline could act as a substitute for nicotine in these situations has not been asked. Male Sprague-Dawley rats were trained to use nicotine (0.4mg/kg subcutaneous) as a conditioned stimulus for access to sucrose (CS+); 36 sucrose deliveries (4 sec each) in 20-min sessions. Conversely, on interspersed days when saline was administered, sucrose was withheld (CS-). Nicotine significantly increased anticipatory food-seeking behavior, measured using dipper entries per sec before the first reinforcer presentation (or equivalent time in CS- sessions). Following this discrimination training, rats were separated into four groups defining the next phase of training: 1) nicotine was maintained as a CS+ (i.e., no change from training); 2) reversal training where nicotine now

serves as a CS- (i.e., saline sessions now predict sucrose access); 3) varenicline replaces nicotine and serves as a CS+; 4) varenicline replaces nicotine in the reversal learning protocol (CS-). Rats that were kept in the drug (nicotine or varenicline) as CS+ groups maintained high levels of goal-tracking to the drug stimulus compared to saline. However, dipper entries per second were lower for rats in the varenicline CS+ group compared to those in the nicotine CS+ group. Early in the reversal learning phase, responding in the now reinforced saline sessions increased to levels controlled by the drug stimuli. With continued reversal training, responding to the non-reinforced drug stimuli began to decrease providing the first evidence of reversal learning in this task.

Disclosures: B.M. Thompson: None. **S.T. Barrett:** None. **J.R. Emory:** None. **C.E. Larsen:** None. **A.M. Peer:** None. **S.T. Pittenger:** None. **R.A. Bevins:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 549.05/KKK25

Topic: G.08. Drugs of Abuse and Addiction

Support: American Diabetes Association Grant 712BS135

NIDA Grant 2R01DA021274

NIDA Grant R15DA040130

Title: Insulin normalizes the strong rewarding effects of nicotine observed in hypoinsulinemic rats.

Authors: *B. CRUZ[1], J. A. PIPKIN[1], R. MARTINEZ[1], C. A. HINOJOSA[1], O. V. TORRES[1,2], A. NAZARIAN[3], L. E. O'DELL[1];
[1]Psychology, Univ. of Texas At El Paso, El Paso, TX; [2]Psychology, San Diego Mesa Col., San Diego, CA; [3]Dept. of Pharmaceut. Sci., Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Introduction: Previous research has demonstrated that hypoinsulinemic rats display enhanced rewarding effects of nicotine. However, it is unclear whether the strong rewarding effects of nicotine observed in these rats are modulated via insulin. To address this issue, the present study examined whether insulin replacement in streptozotocin (STZ)-treated rats would 1) normalize the rewarding effects of nicotine and 2) reverse the alterations in insulin-signaling proteins observed in the brains of STZ-treated rats. **Methods**: A rodent model of hypoinsulinemia was used involving STZ administration, which is a drug that is toxic to the insulin-producing cells of the pancreas. Male rats received STZ (45 mg/kg, sc) or vehicle. Half

of the animals were then implanted with an insulin pellet or received a sham surgery. The rats were then given 23-hour access to nicotine self-administration using an escalating dose regimen (0.03, 0.06 and 0.09 mg/kg/0.1 ml infusion). In a follow up study, western blot analyses were performed in a separate cohort of STZ-treated rats that received insulin replacement. Brain tissue was collected 2 weeks after STZ administration, in order to examine alterations in insulin-signaling proteins at a corresponding time point to our behavioral studies. Tissue was collected from the nucleus accumbens (NAc) for the analysis of the insulin-signaling proteins, IRS-2 and IGF-1Rβ. **Results**: Our behavioral results revealed that insulin replacement normalized the rewarding effects of nicotine in STZ-treated rats. Our protein analysis revealed that the levels of IRS-2 and IGF-1Rβ were increased in STZ-treated rats, and this effect was normalized to control levels in the NAc of rats that received insulin treatment. **Conclusion**: These observations indicate that insulin systems play an important role in modulating the strong rewarding effects of nicotine in hypoinsulinemic rats. Taken together, these results have implications for the development of tobacco intervention approaches in patients with metabolic disorders such as diabetes that suppress insulin signaling.

Disclosures: B. Cruz: None. **J.A. Pipkin:** None. **R. Martinez:** None. **C.A. Hinojosa:** None. **O.V. Torres:** None. **A. Nazarian:** None. **L.E. O'Dell:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 549.06/KKK26

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA 040440

Title: Adolescent rats display stress- and stress+cue-induced reinstatement after cigarette smoke extract or nicotine self-administration

Authors: *C. GELLNER, A. S. ALVELA, H. L. HU, J. D. BELLUZZI, F. M. LESLIE; Pharmacol., Univ. of California, Irvine, Irvine, CA

Abstract: The nearly 90% of people who begin smoking in their teens have a harder time quitting and are more likely to relapse. Therefore, it is important to understand relapse to smoking during this developmental period. To investigate the role of non-nicotine constituents in relapse to smoking, we have established a model of intravenous self-administration comparing cigarette smoke extract (CSE) to nicotine alone. Previous research in our lab has shown that adult male rats that self-administer CSE extinguished more slowly and were more sensitive to

stress-induced reinstatement. We now test the hypothesis that adolescent rats will exhibit similar patterns of behavior. Adolescent rats, aged postnatal day (P) 25, were trained to lever press on a FR5TO20 schedule (1 food pellet per 5 lever presses with a time out period of 20 seconds). After rats reached the reinforced lever press threshold (R=35), they underwent surgery to implant a catheter in their right jugular vein. After three days recovery, rats (P35) began 1-hour drug self-administration sessions with nicotine or CSE (15 µg/kg/infusion nicotine content). Once rats reached stable responding for drug, drug was removed and drug-seeking behavior was extinguished. Extinguished drug-seeking behavior was reinstated with stress, cues, or a combination of stress and cues. Preliminary results suggest that baseline responding for CSE and nicotine groups is similar. When drug is removed, CSE and nicotine seeking rats display similar extinction rates. Adolescents also exhibit similar levels of stress- and stress+cue-induced reinstatement of CSE and nicotine seeking behavior. Therefore, these preliminary findings suggest that the non-nicotine constituents in cigarette smoke do not influence adolescent responding for drug, extinction or reinstatement of drug-seeking behavior.

Disclosures: C. Gellner: None. **A.S. Alvela:** None. **H.L. Hu:** None. **J.D. Belluzzi:** None. **F.M. Leslie:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 549.07/KKK27

Topic: G.08. Drugs of Abuse and Addiction

Support: CIHR

Title: Role of the ventral tegmental area and prefrontal cortex in nicotine-enhanced responding for conditioned reinforcement

Authors: *R. TABBARA[1,3], P. FLETCHER[1,3,2];
[1]Psychology, [2]Psychiatry, Univ. of Toronto, Toronto, ON, Canada; [3]Biopsychology, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Tobacco dependence is a leading cause of preventable death worldwide. Nicotine, the main psychoactive ingredient in tobacco products, possesses several reinforcing properties that may contribute to tobacco dependence. For example, preclinical studies have shown that nicotine enhances locomotor activity, supports self-administration behavior, and induces conditioned place preference. Nicotinic acetylcholine receptors (nAChRs) in the ventral tegmental area (VTA) have been implicated in mediating these reinforcing properties. Nicotine also enhances

responding for sensory cues that function as conditioned reinforcers (CRfs). It is unknown if nAChRs in the VTA are implicated in nicotine-enhanced responding for a CRf. Furthermore, given that responding for sensory cues requires subjects to attend to them, nicotine may enhance their reinforcing value via a prefrontal attentional mechanism. This work examined the role of nAChRs in the VTA and the prelimbic (PrL) area of the prefrontal cortex in nicotine-enhanced responding for a CRf. Male Long-Evans rats were implanted with a bilateral guide cannula aimed at the VTA or the PrL cortex. Next, water-deprived rats received a nicotine injection (0.4 mg/kg; s.c.) prior to 12 sessions of Pavlovian conditioning. Each session consisted of 30 trials wherein a 5-sec tone-light conditioned stimulus (CS) was paired with 0.05 ml of water. Tests of responding for a CRf were then conducted during which presentation of the CS alone (now acting as a CRf) was contingent upon pressing one of two levers (CRf lever). Pressing the other lever had no consequences (NCRf lever). To determine if nAChRs in the VTA or the PrL cortex mediate nicotine-enhanced responding for a CRf, the selective α4β2 nAChR antagonist Dihydro-β-Erythroidine (DHβE; 10 nmol/0.5 μL) was infused into the respective areas prior to a nicotine injection (0.2 mg/kg; s.c.). DHβE infused into the VTA, but not the PrL cortex, attenuated nicotine-enhanced responding for a CRf. Next, to confirm that nAChRs in the VTA mediate nicotine-enhanced responding for a CRf, nicotine (8, 16, or 32 nmol/0.5 μL) was infused into this area. Nicotine infused into the VTA elicited a dose-dependent selective enhancement in responding for a CRf. These findings suggest that nicotine acts within the VTA, but not the PrL cortex, to produce its reinforcement-enhancing effects.

Disclosures: R. Tabbara: None. **P. Fletcher:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 549.08/KKK28

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA040228

Title: Modeling gene x environmental effects in the mouse: the multigenerational phenotypes of the interaction of the rs16969968 SNP with developmental nicotine exposure.

Authors: *H. C. O'NEILL, C. WAGEMAN, S. R. GRADY, M. J. MARKS, J. A. STITZEL; Univ. of Colorado Boulder Inst. for Behavioral Genet., Boulder, CO

Abstract: We have long known of the deleterious effects of developmental nicotine exposure. Recently, a non-synonymous SNP in the a5 nicotinic receptor subunit gene was shown to have

an impact on nicotine addiction; these studies seek to investigate the interaction of this risk allele with exposure to nicotine during development. Female mice with the rs16969968 SNP (hereafter D397 for wild type; N397 for risk allele), were given 100 μg/ml nicotine in a 0.2% saccharin solution 30 days prior to breeding to allow acclimation, and maintained exposure through weaning of pups to reduce the risk of withdrawal. We investigated dopaminergic (DA) release mediated by nicotinic receptor function in the striatum (in isolated synaptosomes) and found DA release is decreased in N397 mice exposed to developmental nicotine (dev nic), suggesting the potential for altered reward. To investigate the rewarding properties of nicotine, we used the two-bottle nicotine choice and conditioned place preference (CPP) paradigms, revealing nicotine aversion in D397 dev nic mice in both studies. N397 dev nic mice, in contrast, consumed the greatest amount of nicotine at high concentrations as compared to all other groups and also showed preference for nicotine in the CPP model. In conjunction with altered DA release, these data combined may suggest increased drive to attain reward in the N397 dev nic mice. We also measured homecage activity and found a significant increase in activity in the N397 mice as compared to the D397 vehicle groups. Both D397 and N397 dev nic mice were significantly more active than their vehicle counterparts during their normal active cycle. All N397 mice also appeared to have sleep disturbances in the homecage setting. Increased activity and possible decreased sleep may contribute to increased nicotine intake if it confers an anxiolytic effect on the N397 mice. We bred D397 and N397 dev nic females to nicotine naïve males creating an F2 generation, and found D397 F2 mice had dramatically increased activity in homecage. Combined, these generational models will allow investigation of structural and functional changes resulting from either direct developmental or germline exposure to nicotine.

Disclosures: H.C. O'Neill: None. **C. Wageman:** None. **S.R. Grady:** None. **M.J. Marks:** None. **J.A. Stitzel:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 549.09/KKK29

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH DA039658

Title: Chronic intravenous nicotine self-administration alters the expression of choroid plexus RNA in rats

Authors: *V. OCHOA, C. D. FOWLER;
Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Tobacco dependence is a leading preventable cause of disease and death worldwide. Given the recent identification of circulating micro-RNAs (miRNAs) in the cerebrospinal fluid (CSF) and documented effects of nicotine on the secretion of protein from the choroid plexus, we sought to investigate whether the expression of RNA becomes altered with chronic intravenous nicotine self-administration. Adult, male Wistar rats were first trained to respond for food pellets in an operant box under a fixed ratio 5, time out 20 sec schedule of reinforcement. Thereafter, rats were implanted with intravenous catheters and randomly assigned into two groups: nicotine or saline (control) self-administration. Following eight days of intravenous self-administration, rats were anesthetized, and CSF was extracted from the cisterna magna. Brains were removed immediately thereafter, and the choroid plexus was microdissected from three distinct anatomical locations in the lateral, third and fourth ventricles. Extracted RNA was analyzed with a microarray and select targets were subsequently verified with RT-qPCR. To further investigate how nicotine may be acting on the choroid plexus, we then assessed the expression of various nicotinic acetylcholine receptor subunits across the ventricular sites. Together, these data support the hypothesis that nicotine acts directly on nicotinic acetylcholine receptors in the choroid plexus to alter RNA expression and release into the CSF.

Disclosures: V. Ochoa: None. **C.D. Fowler:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 549.10/KKK30

Topic: G.08. Drugs of Abuse and Addiction

Title: Comparison of the reinforcing effects of nicotine versus heroin, remifentanil and cocaine with rat intravenous self administration

Authors: *S. L. SMITH, E. JOHNSON, J. SLADE, S. HOLLAND, M. HALLAM, D. HEAL; Renasci Ltd, Nottingham, United Kingdom

Abstract: The relative reinforcing effect of nicotine was compared with three highly abused Controlled Drugs (CDs). They were the opiates, heroin and remifentanil, and the psychostimulant, cocaine. The relative reinforcing effect of the drugs was determined using a progressive ratio (PR) schedule where the rats press operant levers on an escalating response requirement within sessions to receive intravenous drug rewards.
Male Sprague Dawley rats (175-225g on arrival; Charles River, UK) were mildly food restricted and trained to press levers for food rewards. Once responding was stable, animals were implanted with a jugular vein catheter. After nicotine acquisition (0.03mg/kg/infusions [inf], iv)

and saline extinction, a dose-response test was performed for nicotine (0.0075, 0.015, 0.03, 0.06 mg/kg/inf) on fixed ratio 5 (FR-5) schedule in 2 hr sessions. When stable (inf did not vary by >20% over the last 3 sessions) and where each drug dose was positively reinforcing (mean >12 inf/session over the last 3 sessions), the break-point of operant responding was determined in a 4 hr PR session. The break-points for responding for heroin (0.025 mg/kg/inf), remifentanil (0.015 mg/kg/inf) and cocaine (0.29 mg/kg/inf) were determined on a PR schedule. These doses were the most reinforcing on PR schedule in previously performed dose-response tests.
On FR-5, all doses of nicotine were positively reinforcing (inf/session ± SEM: 18.8±1.7 for 0.0075 mg/kg/inf, n=6; 19.8±0.3 for 0.015 mg/kg/inf, n=8; 19.0±0.7 for 0.03 mg/kg/inf, n=8; 18.1±1.4 for 0.06 mg/kg/inf, n=7; 3.7±0.4 for saline; n=8; all doses $p<0.001$ vs. saline).
On a PR schedule, the break-points (lever-presses/infusion) for nicotine were 42.3±0.7 for 0.0075 mg/kg/inf; (n=6), 73.4±14.8 for 0.015 mg/kg/inf (n=8), 48.8±10.0 for 0.03 mg/kg/inf; n=8) and 67.2±10.1 for 0.06 mg/kg/inf; n=7). The break-points for highly reinforcing doses of heroin (61.8±17.7 for 0.025 mg/kg/inf; n=8) and remifentanil (48.1±18.2 for 0.015 mg/kg/inf; n=5) were not different from the most reinforcing dose of nicotine (0.015 mg/kg/inf). The break-point for nicotine was significantly higher than for cocaine (46.6±4.2 for 0.29 mg/kg/inf; n=9; $p<0.05$).
Using an ascending PR schedule, heroin, remifentanil and cocaine were robust positive reinforcers, reflecting their known profiles as highly abused drugs in humans. The relative reinforcing effect of nicotine did not differ from heroin and remifentanil, but was significantly greater than cocaine. In summary, although nicotine is a legally available and widely used drug, it has equivalent or greater reinforcing effect in this model than three Schedule II CDs.

Disclosures: **S.L. Smith:** None. **E. Johnson:** None. **J. Slade:** None. **S. Holland:** None. **M. Hallam:** None. **D. Heal:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA038843

Title: Tobacco flavor additives established as conditioned reinforcers promote nicotine self-administration in rats.

Authors: A. L. SMITH, E. A. WILLIAMS, C. A. BRADLEY, S. G. MALONE, A. K. PATTERSON, E. M. SANDERS, *M. I. PALMATIER;
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Abstract: All tobacco products include flavor additives to enhance flavor and create a unique brand experience. Many additives are conditioned reinforcers (CRs) - they are found in sweet or caloric food and drinks such as candy and ice cream. For example, all Marlboro cigarette products include licorice extract (LE) in the ingredients list. These additives may interact with the effects of nicotine (NIC) to promote tobacco use. For example, NIC is a weak primary reinforcer but potently enhances the reinforcing effect of non-drug CRs, including flavors previously paired with sucrose. The purpose of this study was to investigate whether a ubiquitous cigarette additive (LE) would enhance intravenous NIC self-administration (IVNSA) in rats. We hypothesized that LE would enhance IVNSA if it was established as a CR, but not if it was a neutral stimulus. Male rats (n=34) were randomly assigned to one of two LE groups (CR or Neutral) and one of two LE concentrations (0.1% or 1%, v/v). Rats were allowed to drink solutions containing LE with 20% sucrose (w/v, CR groups) or unsweetened (Neutral groups) for 24 days (40 min per day). Following conditioned reinforcement training, rats were instrumented for IVNSA and allowed to recover. In subsequent IVNSA tests, operant lick responses at two sipper tubes (water or LE) were reinforced with presentations of oral solutions delivered to the sipper tube (0.12 ml). Meeting the schedule of reinforcement on the LE sipper tube delivered the assigned LE flavor (unsweetened) and IV NIC (7.5 ug/kg/infusion). Meeting the schedule of reinforcement on the water sipper tube delivered tap water but did not result in an IV infusion. The schedule of reinforcement was shifted from a fixed ratio of 2 (FR2) to FR5 and FR10 across testing sessions. After responding under the FR10 stabilized, NIC infusions were replaced with 0.9% saline (SAL). During acquisition, only rats in CR groups acquired NIC self-administration; rats in the Neutral groups responded equally at the LE and water sipper tubes. In addition, the stronger LE flavor (1%) was a more robust CR and robustly increased NIC intake, relative to the weaker flavor (0.1%). During SAL substitution, responding at the LE sipper tube declined to the same level as responding at the water sipper tube, but only in the 0.1% CR group. Responding at the LE sipper declined in the 1% CR group, but was statistically higher than responding at the water sipper throughout the SAL substitution phase. In conclusion, flavor additives can interact with NIC to potentiate self-administration and perseveration of operant behaviors after NIC has been removed. These additives may promote exposure and subsequent dependence on tobacco and vapor products.

Disclosures: A.L. Smith: None. **E.A. Williams:** None. **C.A. Bradley:** None. **S.G. Malone:** None. **A.K. Patterson:** None. **E.M. Sanders:** None. **M.I. Palmatier:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH/NIDA T32 DA007027

NIH/NIDA R01 DA031289

Title: Adolescent diet and nicotine treatment interact to affect anxiety-like behaviors in mice

Authors: *L. K. SILVA, D. H. BRUNZELL;
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Abstract: Disease resulting from obesity and cigarette smoking are the leading causes of preventable death in the United States. Adolescents often consume high fat diets and diets with low nutritional content, which have been shown to negatively impact development of the body and brain. Overweight adolescents typically become overweight adults while smokers who initiate use during adolescence report greater consumption and increased difficulty quitting. Additionally, although smokers tend to have lower BMI than non-smokers, heavy smokers are more likely to be obese and obese smokers have more difficulty quitting. Further, high fat diet has been shown to increase β2 subunit containing nicotinic acetylcholine receptors (β2*nAChR), which are known to be necessary for nicotine reward. However, little is known regarding how dietary choices may impact nicotine use vulnerability in adolescents. Nicotine effects on behavior are dose-sensitive, with low doses decreasing anxiety-like behavior, intermediate doses promoting reward and high doses increasing anxiety-like behavior. To study the impact of diet on nicotine reward and anxiety-like behaviors, adolescent C57BL/6J mice (postnatal day (P) 26-47) were maintained on one of three diets: a regular nutrient diet (10% kcal from fat, RND), an identical but reduced vitamin diet (LND), and a high fat (60% kcal from fat, HFD). Mice were tested using doses of nicotine shown to decrease anxiety-like behavior (0.03 mg/kg i.p.) and promote nicotine reward (0.1 mg/kg i.p.) in adult mice. Nicotine conditioned place preference (CPP) was tested from P33-37 and a battery of ethological tests of rodent anxiety-like behaviors was studied from P40-44. Regardless of diet, adolescent mice failed to show CPP at any dose studied. Interestingly, adolescents showed increased anxiety-like behavior at doses shown to promote anxiolysis and reward in adult C57BL/6J mice. RND mice showed elevated anxiety-like behavior in the open field at 0.1 mg/kg i.p. nicotine; HFD mice were left-shifted, showing anxiogenesis behavior at 0.03 mg/kg i.p. nicotine. In the elevated plus maze, LND mice showed reduced time in the open arms at both doses of nicotine, while RND and HFD mice were unaffected by nicotine treatment in this measure. Together, these data suggest that adolescents differ from adults in their response to nicotine and reveal that diet can interact with nicotine to

alter anxiety-like behaviors. Further studies will assess the effect of diet on nicotinic acetylcholine receptor expression and body composition.

Disclosures: **L.K. Silva:** None. **D.H. Brunzell:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: Fundacion Universitaria Konrad Lorenz

Title: Incentive effects of acute or chronic nicotine on acquisition and extinction

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Abstract: Current research has focused on learning and motivational processes for nicotine proposed to be critical for the development and maintenance of tobacco addiction. In particular, nicotine administration has been associated with the enhancement of nonassociative incentive and reinforcing properties of naturally rewarding stimuli. In the present studies, the role of acute and chronic nicotine was assessed on acquisition and extinction, using an autoshaping task with a natural unconditioned stimulus (food pellets). In Experiment 1, subcutaneous acute nicotine (0.4 mg/kg, dose reported as free base) or the appropriate vehicle control was administered during early acquisition or early extinction. Acute nicotine had no noticeable effects on autoshaping acquisition performance, but it retarded autoshaping extinction. In Experiment 2, chronic nicotine (3.6 mg/kg/day; dose reported as free base) or vehicle was administered using mini-osmotic pumps inserted subcutaneously under anesthesia. Contrary to acute nicotine effects, chronic nicotine enhanced autoshaping acquisition performance, but had no noticeable effects on extinction performance. Together, these findings suggests a differential effect of nicotine on autoshaping acquisition and extinction depending upon the type of nicotine administration, acute or chronic. As chronic, but not acute, nicotine administration is associated to upregulation of nicotinic cholinergic receptors, these findings also suggests differential brain mechanisms underlying the specific effect of chronic nicotine on acquisition. Future studies on this differential effect may help understand the mechanisms underlying the complex and varied incentive and reinforcing effects of nicotine on natural stimuli.

Disclosures: **L.A. Ortega Murillo:** None. **M.R. Papini:** None. **D. Ramirez:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA grant R21DA040195

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Title: Effects of genetic manipulation of metabotropic glutamate receptor 7 on nicotine self-administration in rats and mice

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Abstract: The reinforcing properties of nicotine are associated with increased glutamate transmission in the mesocorticolimbic circuit. Decreasing glutamate transmission by targeting metabotropic glutamate receptors (mGluRs), such as mGluR2/3 and mGluR5, inhibited nicotine taking. Our recent studies showed that activation of mGluR7 by the only commercial available agonist AMN082 attenuated nicotine reinforcement. However, findings indicated that some effects of AMN082 were not mediated by mGluR7, but rather by monoamine reuptake inhibition induced by an AMN082 metabolite, making the AMN082-induced effects difficult to interpret. The present study was aimed to assess the role of mGluR7 in nicotine reinforcement in mGluR7 null mutant mice and in mGluR7 overexpression rats. We found that mGluR7 null mutant mice showed increased nicotine self-administration, but had no change in food self-administration, compared to their wildtype littermates. AMN082, at middle dose (3 mg/kg), inhibited nicotine self-administration in wildtype, but not mGluR7 gene knock, mice. However, high dose of AMN082 inhibited nicotine taking in both wildtype and gene knock mice. Moreover, overexpression of mGluR7 in the ventral tegmental area (VTA), an important brain area involved in drug addiction, decreased nicotine self-administration, but had no effect on food-self-administration in rats. These findings in genetic manipulation animals are consistent with those of our previous findings with pharmacological manipulations, further suggesting the critical role of mGluR7 in nicotine reinforcement. Therefore, mGluR7 may be a promising target for the treatment of nicotine addiction.

Disclosures: **X. Li:** None. **A. Markou:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); San Diego Instruments. F. Consulting Fees (e.g., advisory boards); Omeros, Inc..

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA032543

NIH Grant DA039658

Title: ChAT-BAC-Cre and ChAT-IRES-Cre mouse lines exhibit differential patterns of intravenous nicotine self-administration

Authors: A. LOEFFLER, E. CHEN, J. P. FOWLER, F. NOSRATI, N. P. GRIMES, *C. D. FOWLER;
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Abstract: The recent development of transgenic mouse lines expressing cre in a cell-specific manner, along with advances in engineered viral vectors, has permitted increased investigation into neural circuit function. However, emerging evidence has indicated that some transgenic mouse lines may introduce limitations into experimentation based on the method of development. For instance, it has been recently reported that ChAT-ChR2-EYFP transgenic mice that express channelrhodopsin under the ChAT promoter exhibit increased baseline cholinergic tone (Kolisnyk et al., 2013, *J Neurosci*). In the current investigations, we sought to characterize both the ChAT-BAC-Cre line created with the bacterial artificial chromosome method (GENSAT) and the ChAT-IRES-Cre line generated with the internal ribosome entry site method (Jackson Laboratory). Adult male and female mice were first trained in the food self-administration paradigm under a progressive fixed-ratio schedule to achieve a fixed-ratio 5, time out 20 sec schedule of reinforcement. Both lines exhibited no deficits in the acquisition of food responding across sessions, indicating an ability to learn and perform an operant task similar to their wildtype littermate controls. Thereafter, mice were surgically implanted with intravenous catheters into the right jugular vein. After a recovery period and reinstatement of food responding, the mice were transitioned to intravenous self-administration at the 0.03 mg/kg/infusion training dose of nicotine. At this dose, ChAT-BAC-Cre mice quickly attenuated their level of responding and did not sustain consistent nicotine self-administration behavior,

whereas ChAT-IRES-Cre mice exhibited sustained nicotine self-administration at a level similar to wildtype controls. Given the dense expression of acetylcholine in the medial habenula-interpeduncular pathway and the role of this structure in mediating nicotine aversion, we then investigated whether the mouse lines differed in their expression of the vesicular acetylcholine transporter and cre protein in the medial habenula and interpeduncular nucleus. Together, these data suggest that ChAT-BAC-Cre, but not ChAT-IRES-Cre, mice exhibit altered cholinergic signaling that results in increased sensitivity to the aversive properties of nicotine. As such, investigations with these mice should proceed with caution in consideration of this altered phenotype.
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Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA040440

Title: Role of a3b4 nicotinic acetylcholine receptors in drug- primed reinstatement of Cigarette Smoke Extract (CSE)- and nicotine- seeking behavior

Authors: *D. REYNAGA[1], N. ZAVERI[2], F. LESLIE[3];
[1]Univ. of California Irvine, Irvine, CA; [2]Astraea therapeutics, Mountain View, CA; [3]Univ. of California, Irvine, Irvine, CA

Abstract: Cigarette smoking is difficult to treat and the majority of those who try to quit relapse within the first year. Many smoking cessation therapies fail at the clinical phase of drug development. One explanation for this may be that the majority of preclinical tests use nicotine alone, ignoring ~8,000 constituents also found in tobacco smoke. In prior studies, we have shown that rats that self-administered cigarette smoke extract (CSE), an aqueous solution containing nicotine and other smoke constituents, were more sensitive to stress- induced reinstatement of drug seeking behavior than were those that self-administered nicotine alone (Costello et al. 2014). Furthermore, AT-1001, a functional antagonist of α3β4 nicotinic acetylcholine receptors (nAChRs), was less effective in blocking self-administration of CSE than nicotine alone. We have now examined whether rats that self-administer CSE are also more

sensitive to drug- primed reinstatement than those that worked for nicotine alone, and whether AT-1001 has differential effects on drug- primed reinstatement in the two groups. Upon stable self-administration and extinction of CSE or nicotine responding (15 μg/kg/infusion nicotine content, i.v.), all animals were tested for reinstatement in a randomized design with the following 5 conditions: cues alone, nicotine priming (0.15mg/kg; i.p), CSE priming (0.15mg/kg nicotine content; i.p), and both drug priming conditions with cues. To test attenuation of reinstatement, AT-1001 (0, 0.75, 1.5, 3 mg/kg; s.c.) was given before the test followed by reinstatement testing with a priming dose of the previously self-administered drug (CSE or nicotine; 0.15 mg/kg nicotine content; i.p) paired with cues. We found that both animals that self-administered CSE or nicotine reinstated when drug priming was paired with cues. However, only animals that self-administered CSE reinstated when drug priming was given without cues. Furthermore, animals that self-administered CSE reinstated more robustly than animals that self-administered nicotine alone when given a priming dose of nicotine. AT-1001 dose dependently reduced drug- primed reinstatement both in animals that self-administered CSE and nicotine, with a trend toward higher potency in animals that self-administered nicotine. The results suggest that the non-nicotine constituents in CSE, contribute to the increased propensity for reinstatement, likely through nAChR sensitization. The attenuation of nicotine- and CSE- seeking behavior by AT-1001 suggests that α3β4 nAChR functional antagonism may be a suitable treatment approach to reduce nicotine and CSE craving during smoking cessation treatment.

Disclosures: D. Reynaga: None. **N. Zaveri:** None. **F. Leslie:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: NRF-2013R1A1A1057712

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Title: Bimodal modulation of social factors on nicotine aversion in rats: Focus on sex difference, pair housing and oxytocin

Authors: H. LEE, M. JANG, *J.-H. NOH;
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Abstract: Tobacco smoking is controlled in diverse social circumstances. Social therapy is generally used to stop smoking, while peer interaction is insisted as a crucial factor which triggers initial tobacco use in adolescent. To determine the roles of social factors on nicotine dependence, we compared single and pair housed rats in dose escalating voluntary oral nicotine consumption. There were no differences in body weight and food intake among all assigned rats. Importantly, pair housed female rats showed alleviated nicotine consumption for both high and low dose of nicotine solution. On the other hand, pair housed male rats showed only reduced consumption in high dose nicotine solution compared to single housed rats. Moreover, in male rats, aversive response to nicotine was contrastively disappeared in pair housed rats in low dose of nicotine consumption. To demonstrate the other social factor which can mitigate nicotine aversion in male rats, we observed oxytocin-injected male rats on voluntary oral nicotine consumption against nicotine solution. As a result, oxytocin-injected rats showed enhanced nicotine consumption compared to saline-injected rats. Taken together, it provides a widen perspective on social interaction as a therapeutic strategy on nicotine addiction related behaviors. We suggest that revealing distinct roles of behavioral and physiological social factors have fascinating potentials for inoculating nicotine addiction. (Supported by NRF-2013R1A1A1057712 and NRF-2014R1A2A2A04007391)

Disclosures: **H. Lee:** None. **M. Jang:** None. **J. Noh:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Support: NIH Grant DA034389

Nebraska DHHS - LB506 2015 "Nicotine Enhancement of Alcohol Seeking"

Title: Nicotine alters demand for alcohol reinforcement in male and female Sprague-Dawley and Wistar rats.

Authors: *S. BARRETT, B. M. THOMPSON, S. T. PITTENGER, C. E. LARSEN, J. R. EMORY, R. A. BEVINS;
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Abstract: Nicotine and alcohol dependence are highly correlated: up to 80% of alcohol-dependent persons in the US smoke regularly, and risk of alcohol dependence is four times

higher among people who are nicotine dependent. Additionally, differences in the prevalence and nature of nicotine and alcohol dependence exist between the sexes. Women are less likely to attempt smoking cessation and more likely to relapse after quitting. Women also show greater sensitivity to the intoxicating effects of alcohol, slower alcohol metabolism, and greater risk for adverse health consequences of alcohol use. Understanding the behavioral processes that underlie alcohol and nicotine dependence and their high comorbidity will require consideration of the multifaceted causes that include an individual's sex.Increasing evidence suggests that the reward-enhancing effects of nicotine synergistically enhances behavior directed at obtaining other rewards, and this reward-enhancement effect holds promise in deciphering the mechanisms that drive nicotine dependence. That is, nicotine administration that occurs in the same context as the reception of other rewarding stimuli or may enhance their rewarding effects, and this enhancement may drive motivation to abuse nicotine. Recently, we demonstrated that experimenter-administered nicotine alters alcohol consumption in Wistar rats and that this effect depends in part on the response cost to obtain alcohol reinforcement. Briefly, 0.4 mg/kg nicotine decreased alcohol intake when alcohol delivery was relatively affordable but increased alcohol consumption when alcohol presentation was relatively expensive. Additionally, sex differences were observed in alcohol reinforcement under saline-control conditions, and nicotine administration modulated these differences. In the present study, we assessed the effects of nicotine across a range of doses (0.05, 0.1, 0.2, and 0.4 mg/kg) on 15% ethanol self-administration in male and female Sprague-Dawley and Wistar rats. Rats we trained to self-administer ethanol using an extended sucrose fading procedure to ensure high intake. Subsequently, the response cost (FR schedule) per 0.1 mL delivery of 15% ethanol was increased over blocks of 10 sessions, and ethanol consumption was assessed as a function of response cost. A behavioral-economic reinforcer-demand model was applied to the data and separate demand curves were constructed for each nicotine dose between sex and strain conditions. The effects of nicotine to alter ethanol reinforcement value were analyzed using the Q_0 and *essential value* parameters generated by these demand curves.

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Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: Ministry of Food and Drug Safety (14182MFD977), Korea

Title: Cigarette smoke condensate increases glutamate releases in the dorsal striatum and behavioral changes in locomotor activity and rearing

Authors: I. RYU[1], J. KIM[1], S. SEO[1], J. YANG[1], J. OH[2], K. LEE[3], S. YOON[4], J.-W. SEO[4], *E. CHOE[1];
[1]Pusan Natl. Univ., Pusan, Korea, Republic of; [2]Fisheries Sci., Busan, Korea, Republic of; [3]Inhalation Toxicology Res. Ctr., Jeongeup, Korea, Republic of; [4]Res. Ctr. for Safety Pharmacol., Daejeon, Korea, Republic of

Abstract: Nicotine is a key compound having reinforcing effects on initiation and maintenance of tobacco dependence through potentiation of dopaminergic and glutamatergic neurotransmission. Non-nicotine alkaloids in tobacco also contribute to tobacco dependence by activating cholinergic system. However, glutamatergic neurotransmission in the dorsal striatum associated with psychomotor sensitization in response to cigarette smoking has not been determined. In this study, we investigated alterations in glutamate response in the dorsal striatum related to behavioral alterations after repeated administration of cigarette smoke condensate (CSC). The results demonstrated that repeated administration of three types of CSCs (3R4F, KCP-A and KCP-B) significantly increased extracellular glutamate concentrations as compared with repeated nicotine administration alone. Paralleled to the hyperactivity of the glutamate response, repeated administration of these CSCs occurred a prolonged hypersensitization of psychomotor activity including locomotor activity and stereotypy movement. These findings suggest that non-nicotine constituents in cigarette smoke synergistically upregulate the reinforcing effects of nicotine on glutamatergic response in the dorsal striatum, which seems to be a necessary neurochemical event leading to tobacco dependence.

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Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: Virginia Foundation for Healthy Youth

Virginia Youth Tobacco Programs/VCU

Title: Nicotine consumption is strongly influenced by social interactions in male, but not female, adolescent mice

Authors: A. D. HUDSON, R. L. MURPHY, *K. J. FRYXELL;
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Abstract: Studies of human teenagers have shown that social isolation and emotional stressors increase the likelihood of smoking initiation. Early smoking initiation and/or symptoms of emotional stress, including depression and anxiety, are correlated with increased rates of smoking. In rodent models, previous studies have shown that unpredictable chronic mild stress increased the sensitivity of adolescent male rats to the lowest nicotine doses, but reduced their preference for higher nicotine doses. In another study, chronic isolation (a form of mild stress) of adolescent mice from weaning to adulthood did not increase subsequent cocaine self-administration. However, the rewarding properties of adolescent peer interactions differed between adolescent male and female rats, and prior isolation dramatically increased social reward only in adolescent males, as shown by an assay of conditioned place preference. Here we show that social isolation (vs. pair housing) of adolescent male mice during a test of voluntary oral nicotine consumption dramatically increased the amounts of nicotine chosen by adolescent male C57BL/6J mice. Adolescent female C57BL/6J mice also increased their nicotine consumption under conditions of social isolation (vs. pair housing), but the increase was much smaller than males. Moreover, isolated adolescent males showed significantly elevated nicotine consumption immediately after their first access to nicotine, suggesting that social reward (and/or anxiety) modified drug-seeking on a short-term basis, particularly in adolescent males. These observations, particularly the effectiveness of social isolation only when it occurs simultaneously with prolonged access to nicotine, is consistent with a “reward deficit” model in which the rewarding properties of adolescent social interactions partially substitute for (and hence reduce the need for) nicotine reward. It is also consistent with previous observations on adolescent male rats, showing that nicotine injections interacted synergistically with the rewarding properties of social interactions in a conditioned place preference assay, but only if those two treatments were delivered simultaneously.

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Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Program#/Poster#: 549.21/KKK41

Topic: G.08. Drugs of Abuse and Addiction

Title: Modeling habitual nicotine-seeking in rats

Authors: ***A. J. LOUGHLIN**[1,2], K. COEN[1], D. FUNK[1], A. D. LÊ[1,2,3];
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Abstract: Addiction to nicotine, usually in the form of smoked tobacco, can persist in spite of repeated attempts to quit. A possible reason for this is that repeated voluntary intake of nicotine may lead to a rapid transition from goal-directed to habitual behavioral control. Although accelerated habit formation with self-administered ethanol and cocaine has been previously demonstrated, this phenomenon has not been extensively studied with nicotine. To examine the liability of nicotine self-administration to become habitual, male rats were allowed to lever-press for intravenous nicotine for 10 consecutive days, under a fixed-ratio 1 (FR-1) schedule. As a control, these same rats also lever-pressed for oral saccharin solution in separate daily sessions. In the first experiment, nicotine or saccharin were devalued by pairing with the aversive agent lithium chloride; this treatment specifically reduced responding for both nicotine and saccharin in subsequent reacquisition tests, indicating both rewards could be successfully devalued. In the second experiment, the contingency between lever pressing and delivery of nicotine or saccharin was degraded during six sessions, across which lever responding declined for non-contingently delivered saccharin, but not for non-contingently delivered nicotine. Prior to reacquisition testing (Experiment 1) but following contingency degradation (Experiment 2), animals were also tested under extinction conditions. During these extinction tests, responding on the lever previously associated with saccharin delivery was reduced by both devaluation and contingency degradation, suggesting that the lever pressing for saccharin was goal-directed. In contrast, pressing on the lever previously associated with nicotine delivery was not affected by either devaluation or contingency degradation, suggesting that responding for nicotine was habitual. These results suggest that 10 days of FR-1 training produced habitual responding for nicotine, but goal-directed responding for saccharin, and therefore demonstrate that operant responding for intravenous nicotine may become habitual more rapidly than responding for oral saccharin. This augmented development of habitual responding for nicotine may help to account for the persistence of tobacco use.

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Poster

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Disgust (but not fear) graphic warning labels reduce EEG-indexed cognitive processing of smoking-related visual cues

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Abstract: Smokers exhibit cognitive biases in the processing of smoking-related stimuli that are thought to facilitate the detection of smoking cues and play a perpetuating role in addiction. Graphic warning labels (GWLs) are being added to cigarette packaging in the hope that they will reduce smoking; yet, the ability of these images to discourage continued tobacco use is still being debated. Historically, research has assessed the impact of GWLs using attentional (eye tracking) paradigms and done so in images that mix fear- and disgust-inducing elements. The current study had smokers view categorized fear and disgust images while an EEG was recorded. It was hypothesized that both the fear and the disgust GWLs would distract and disrupt attention-related cognitive processing of the smoking cue, as indexed by reduced P3 and LPP activations, but that disgust's effects would be larger.

In the current study, 61 smokers participated in an oddball paradigm consisting of 3 counterbalanced blocks of visual stimuli while an EEG was recorded. Each block (100 trials each) used the same positive smoking cue as the oddball image (20%) while the frequent image differed in each block: neutral, GWL-fear, or GWL-disgust image. The P300 (P3) and the Late Positive Potential (LPP) components of the event-related potential (ERP), which index the deployment of attentional resources to motivationally relevant stimuli such that they are enhanced by motivationally relevant stimuli and reduced when attention is directed elsewhere, were assessed.

Analysis revealed that, as expected, the P3 (250-600ms) and the LPP (500-1000ms) varied between stimuli blocks at Cz. Somewhat consistent with expectation, post-hoc comparisons revealed that the disgust GWLs led to reduced area activation in the P3 and LPP compared to the fear GWLs and neutral blocks; there were no differences in P3 or LPP between the neutral and fear GWL blocks. These results extend prior work by separating fear and disgust elements in GWLs and in providing preliminary support for the suggestion that GWLs on cigarette packaging may reduce attention to smoking related cues. Disgust (but not fear) GWLs appear to disrupt attention-related cognitive processing of smoking cues and may represent a fruitful target in cessation efforts.

Disclosures: **J. Cochran:** None. **M.J. Lee:** None. **N. Walker:** None. **R. Kydd:** None. **N. Consedine:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

Location: Halls B-H

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Topic: G.08. Drugs of Abuse and Addiction

Support: K01 DA030445

R21 DA039393

Title: Paternal nicotine self-administration is associated with increased nicotine taking and anxiety-like behaviors in offspring

Authors: *J. J. MAURER, C. A. TURNER, J. D. WOLFHEIMER, C. A. PIERCE, M. E. WIMMER, H. D. SCHMIDT;
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Abstract: Human epidemiological studies indicate that children of parents who smoke tobacco have a higher incidence of developing nicotine dependence, cognitive deficits and anxiety. These findings suggest that nicotine may reprogram the germline epigenome to produce aberrant behavioral phenotypes in subsequent generations. The goal of this study is to establish a novel animal model of the inter-generational effects of voluntary preconceptional nicotine taking. Male Sprague Dawley rats were allowed to self-administer nicotine (0.03 mg/kg/infusion) on a fixed-ratio 1 (FR1) schedule of reinforcement for 60 consecutive days. Each nicotine-experienced rat was paired with a yoked saline control rat that received the same number and temporal pattern of infusions. Following nicotine self-administration, nicotine-experienced and yoked saline control rats were allowed to mate with drug-naïve dams. At P60, acquisition of nicotine self-administration was assessed in the drug-naive offspring (F1 generation). Both female and male nicotine-sired offspring self-administered significantly more nicotine than saline-sired offspring. These effects were specific to nicotine, as sucrose taking and cocaine self-administration were not altered in the offspring of nicotine-exposed sires. Memory formation and anxiety-like behaviors were measured in separate cohorts of F1 littermates. Our preliminary results indicate that male and female nicotine-sired offspring had increased anxiety-like behavior in the novelty-induced hypophagia (NIH) and shock-probe defensive burying (DB) paradigms compared to saline-sired controls. Taken together, these data are consistent with human epidemiological studies and indicate that voluntary paternal nicotine taking produces heritable addiction-like and anxiety-like phenotypes. To identify potential mechanisms underlying the inter-generational effect of nicotine, future studies will profile transcriptomes in brain regions known to regulate the effects of nicotine on behavior as well as epigenetic modifications within gametes. Identifying the mechanisms underlying transmission of enhanced vulnerability nicotine and

mood disorders could aid in the development of novel pharmacotherapies for individuals at risk for developing chronic smoking behavior and mental disorders.

Disclosures: **J.J. Maurer:** None. **C.A. Turner:** None. **J.D. Wolfheimer:** None. **C.A. Pierce:** None. **M.E. Wimmer:** None. **H.D. Schmidt:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

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Title: Sex differences in stress-induced reinstatement of nicotine seeking in a mouse conditioned place preference model

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Abstract: Clinical and epidemiological research indicate that stress contributes to smoking relapse and that women are particularly vulnerable to this effect. Further, research in human smokers has shown that targeting the noradrenergic system with guanfacine, an alpha2-adrenergic agonist, can reduce stress-precipitated smoking in a laboratory setting. Therefore, pharmacological interventions that can successfully target this stress response may be especially beneficial for women in reducing relapse to smoking. Preclinical models of behaviors related to nicotine addiction have demonstrated stress-induced reinstatement of nicotine seeking and attenuation of stress-induced reinstatement through noradrenergic manipulations. Furthermore, sex differences in catecholaminergic and cholinergic systems and in nicotine-seeking behavior have been observed in rodent models. However, sex differences in stress-induced reinstatement of nicotine seeking, specifically, or its modulation using adrenergic agents has not been studied in rodents. We developed a behavioral protocol based on the conditioned place preference (CPP) model of drug seeking to investigate this phenomenon in C3H/HeJ mice. We administered nicotine (0.5, 0.75, or 1.0 mg/kg) subcutaneously during the conditioning phase and observed a dose-dependent preference for the nicotine-paired chamber that differed in male and female mice. We also observed sex differences in the preference for the nicotine-paired chamber after a period of abstinence. Both sexes subsequently demonstrated successful extinction learning after

repeated exposure to the CPP chambers in the absence of nicotine, and reinstatement of preference for the nicotine-paired chamber following a forced swim stress. Interestingly, our preliminary data suggest that guanfacine (0.15 mg/kg, intraperitoneal) blunts this stress-induced reinstatement in a sex-dependent manner. Therefore, this behavioral paradigm may be a valuable tool in studying nicotine-seeking behavior in male and female mice, especially in response to stress. Genetic, molecular, and cellular techniques can then be applied in this paradigm to investigate the neurobiological mechanisms underlying sex differences in stress-induced reinstatement of nicotine seeking and its attenuation with adrenergic agents.

Disclosures: **A.M. Lee:** None. **Y.S. Mineur:** None. **M.R. Picciotto:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Topic: G.08. Drugs of Abuse and Addiction

Support: PRODEP (PTC-761) to CJP

Title: Activation of locomotor activity by imposed inhalation of nicotine

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Abstract: The establishment of animal models has contributed to advances in the understanding of the neural basis of nicotine addiction. These models include self-administration and experimenter-administration (imposed), through intraperitoneal and subcutaneus pathways. These techniques imply an important manipulation of the subjects causing stress, besides, distant from the typical way of nicotine intake in humans by inhalation. Administered by either method, it is expected that the nicotine acts over neural regions of the limbic system, related to drugs intake. The activation of this circuit is behaviourally expressed in an increase of locomotor activity and some somatic signals, depending on the dose. In addition, novel drug administration methods have been reported, as imposed nebulization of methamphetamine. However, this latter has not been explored using other drugs as nicotine, so required concentrations to get the behavioral effects observed under traditional methods are unknown. This is relevant since nebulization protocol simulates the human intake condition. The goal of this study was to

standardize and validate the imposed nebulization of nicotine as an effective nonivasive protocol of intake. Wistar male rats were housed under light-dark conditions 12-12 h, food and water intake ad libitum. Locomotor activity was evaluated using the open field test at eight experimental conditions: 1) 0.35mg/kg of nicotine s.c., 20 min nebulization of: 2) distilled water, 3) 0.5 mg/ml, 4) 1.0 mg/ml, 5) 1.5 mg/ml, 6) 2.0 mg/ml, 7) 2.5 mg/ml, and 8) 3.0 mg/ml of nicotine solution. Results indicate that locomotor activity increased in subjects nebulized with 2.5mg/ml nicotine solution similar to that reported using subcutaneus administration of 0.35mg/kg of this drug. We conclude that this dose is useful for the imposed nebulization protocol to study the effects of nicotine in a more effective model that simulates human condition of intake.

Disclosures: **R.C. Zepeda:** None. **T. Molina:** None. **G. Roldán-Roldán:** None. **Á. De Felipe:** None. **E. Meza:** None. **M. Caba:** None. **C. Juárez-Portilla:** None.

Poster

549. Nicotine: Reinforcement, Seeking, and Reinstatement

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Program#/Poster#: 549.26/KKK46

Topic: G.08. Drugs of Abuse and Addiction

Title: Chronic stress enhances nicotine-seeking behavior in an animal model of addiction

Authors: *J. J. CORTRIGHT, A. MILLER, E. ANDERSON, H. KLIMEK, A. JANKE, T. HARMAN;
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Abstract: Drug addiction is a major public health and serious economic concern in the United States costing taxpayers billions of dollars annually. Experimental evidence shows that exposure to stress is not only a factor in the development of addiction; but also a trigger for drug relapse, or reinstatement. As tobacco use has been linked to a number of cancers and represents the leading cause of preventable death in the United States, elucidation of the effects of stress on nicotine-seeking behavior and relapse is critical. A critical role of chronic stress in the compulsion to seek tobacco and other nicotine delivering products has long been suspected. Although many studies have provided compelling evidence for a role of chronic stress in the enhanced sensitivity to cocaine-seeking behavior and relapse, few have assessed the contribution of chronic stress on nicotine-seeking behavior. In fact, stress induced cross-sensitization to nicotine remains controversial. Additionally, there have been no studies investigating the effects of chronic stress on nicotine-seeking relapse, or reinstatement. Thus, these experiments assess the ability of repeated exposure to variable stress to augment nicotine-seeking behavior and

relapse in an animal model of drug addiction. Male Long-Evans rats were exposed to variable stress that consisted of the exposure to different stressors once a day in random order for 20 days. During this period the control group was left undisturbed except for cage cleaning. Rats were allowed to self-administer nicotine (0.03 mg/kg/infusion) under fixed ratio schedules of reinforcement across 15 consecutive daily sessions. Responding under a progressive ratio schedule of reinforcement was assessed over the following six daily sessions. This schedule allows for break points to be analyzed, a measure that reflects the motivation to self-administer nicotine. Following up to 20 days of extinction training, rats were tested for nicotine-seeking behavior reinstatement by a non-contingent injection of nicotine (0.4 mg/kg, IP). Rats exposed to chronic stress acquired nicotine self-administration at a faster rate relative to controls, exhibited enhanced motivation to obtain the drug, and were more resistant to nicotine extinction. Further, exposure to chronic variable stress led to enhancements in nicotine-primed reinstatement, or relapse. Collectively, these findings indicate that chronic stress can enhance the motivational effects of nicotine.

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Poster

550. Executive Function: In Vivo Recording and Functional Study

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Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI 20020015

KAKENHI 21240024

Title: Persistent activity of prefrontal neurons as a source of confidence in working memory

Authors: *A. TANAKA, S. FUNAHASHI;
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Abstract: The prefrontal cortex has been thought of as an important structure for working memory processes. Previous neurophysiological studies have repeatedly shown that prefrontal neurons exhibit stimulus-selective persistent activity during a period after the offset of a sensory stimulus, which has been considered as a neural correlate of the short-term maintenance of information. In the present study, we examined whether and how this prefrontal persistent activity is related to subjective confidence in working memory. Two macaque monkeys were

trained on a modified oculomotor delayed response task. In this task, the subject was required to remember the location of a visual cue toward which it had to make a saccade after a several-sec delay period as a memory test. Task difficulty was controlled by varying the number of distractors that were presented during the delay and response periods. After the delay period but before the response period, the subject was sometimes allowed to choose to take or decline the memory test and sometimes forced to take the test. A correct response to the test was rewarded with a drop of juice, while no reward was given for an incorrect response. Declining the test led to a simple visually guided saccade task, but only 30 to 50% of correct saccades were rewarded. Under these conditions, monkeys' performance on the memory test was significantly higher when they chose to take the test than when they were forced to do so, which suggests that the monkeys tended to decline the test when the cued location was not remembered. While the monkeys were performing this task, a number of prefrontal neurons exhibited persistent activity that was tuned to particular spatial locations during the delay period. Importantly, the spatial selectivity of this delay-period activity was significantly weaker when the monkeys chose to decline the memory test than when they chose take the test. This reduction in spatial selectivity was not due to a decrease in responses to preferred directions but due to an increase in responses to nonpreferred directions. In other words, the monkeys tended to decline the memory test in trials in which the responses of neurons whose preferred directions differed from the cued location were failed to be inhibited. Thus, the quality of spatial information represented by prefrontal neuronal activity was related to the subject's choice to take or decline the memory test. We propose that the spatially selective persistent activity of prefrontal neurons serves as a source of confidence in visuospatial working memory and that this memory-related signal would be transmitted to a neural circuit that is responsible for computing the degree of confidence.

Disclosures: A. Tanaka: None. **S. Funahashi:** None.

Poster

550. Executive Function: In Vivo Recording and Functional Study

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Topic: H.01. Animal Cognition and Behavior

Support: DFG grant NI 618/3-1

Title: The neural representation of large numerosities in the corvid nidopallium caudolaterale

Authors: H. M. DITZ[1], *A. NIEDER[2];
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Abstract: Number recognition is widespread throughout the animal kingdom, and found, for instance, in insects, fish, amphibians, and birds. So far, most studies exploring the neural code of number investigated non-human primates. It remains elusive how large numerosities beyond five items are represented in animals lacking a prefrontal cortex. In this study, we investigated how large numerosities are represented in birds with independently developed endbrain structures that enable intelligent behavior. The telencephalic nidopallium caudolaterale (NCL) is considered to be the avian homologue of the prefrontal cortex. We trained two Carrion crows (Corvus corone corone) to differentiate dot arrays with 1 to 30 dots (controlled for total dot area and dot density) in a delayed match-to-sample task while we recorded single cells in the NCL. We recorded a total of 325 neurons. About a fifth of those neurons were significantly tuned to numerosity during sample presentation and during the 1-sec memory delay. Neural preference for a certain numerosity was distributed over the whole shown numerosity range. The neuronal tuning curves exhibited a peak in activity for their preferred numerosity and a systematic drop-off in activity the further the shown numerosity was numerically distant to the preferred one (distance effect). In addition, the tuning functions grew broader with increasing preferred numerosity (magnitude effect). Analysis of the tuning functions revealed that both small and large numerosities are both best described by a logarithmic scale as predicted by the Weber-Fechner law. The existence of the distance and magnitude effects together with logarithmic number scaling indicates the presence of an underlying analogue magnitude system to encode numerosities.

Disclosures: H.M. Ditz: None. **A. Nieder:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH 5R37MH087027

Title: Laminar-specific coding of working memory in frontal cortex

Authors: *A. BASTOS, R. LOONIS, E. K. MILLER;
Picower Inst. For Learning and Memory, Cambridge, MA

Abstract: All cortical areas have some degree of laminar anatomical organization, which is characterized by different local and long-range inputs and outputs, expression levels of different molecular markers, and cell types. These distinctions have inspired many theories about the putative functions of different layers, but little physiological data exists to support these claims.

In particular, the role of the different cortical layers for cognition remains relatively unexplored. To address this, we recorded spike and LFP data from the frontal cortex (area PMd and SEF) of a macaque monkey with multiple 16 and 24 channel linearly-spaced multicontact probes as the animal performed a visual working memory (WM) task. As previously observed in visual cortex, LFP power in gamma frequencies (40-100 Hz) was strongest in superficial layers (L1-3), and alpha frequencies (8-12 Hz) predominated in deep layers (L5-6), suggesting some degree of functional compartmentalization by layers. We next examined the role of different frequency bands and layers for encoding WM information during the delay period of the task. We found that brief, punctate bursts of gamma-band activity in superficial layers reliably encoded the spatial position held in WM, but deep layers and other frequencies carried either very little or no information about WM contents. Deep and superficial layers synchronized their LFPs at sub-gamma frequencies, with spectral peaks in the alpha and beta frequency (~20-25 Hz) bands. Granger causality analysis revealed that this alpha-beta interaction was primarily unidirectional, with deep layers driving superficial. Finally, cross-frequency coupling analysis showed that the phase of delay period alpha oscillations in deep layers modulates the gamma amplitude of superficial layers. These analyses suggest a modulatory role for deep layers in WM maintenance, and an active role for superficial layers which encode WM contents in information-rich bursts of high-frequency gamma activity.

Disclosures: **A. Bastos:** None. **R. Loonis:** None. **E.K. Miller:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: EH15-098

Title: fMRI assessment of anesthesia-induced learning deficiency

Authors: ***D. P. AKSENOV**, M. MILLER, L. LI, A. WYRWICZ;
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Abstract: Each year approximately 6 million children in the USA alone undergo anesthesia in the course of surgeries and other diagnostic procedures. A growing body of literature has indicated that early exposure to general anesthesia can affect neuronal development, leading to deficits in learning and memory. It was found that children who underwent anesthesia were more than twice as likely to exhibit behavioral/developmental deficits in young adulthood. Animal

studies enable a more well-controlled analysis of anesthesia-related effects and allow for more precise comparisons between anesthetized subjects and controls. A variety of animal models have been used to study the effects of inhaled anesthetics and have revealed associations between early anesthesia exposure and developmental pathologies including increased cell apoptosis, defects in myelination, hippocampal and cortical cell loss and disruption of associative learning in adult subjects. The functional changes in the brain associated with such impairments have not been directly characterized. Previously, we have used blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) to examine the learning-related functional changes that occur during eyeblink classical conditioning (ECC) in awake, behaving rabbits. In ECC the subject learns to associate a neutral conditioned stimulus (CS) with a behaviorally salient unconditioned stimulus (US). fMRI allowed us to evaluate the functional state of the sensory system before learning, and together with ECC provides a non-invasive, easily quantifiable approach for characterizing the impact of anesthesia on learning. This study evaluated the effects of anesthesia exposure during infancy on learning-induced functional activity in the cortex during young adulthood. Infant rabbits were exposed to isoflurane anesthesia using a common surgical protocol and then received training with a trace ECC paradigm at three months of age. fMRI experiments were performed to measure the BOLD response to the whisker vibration CS before and after learning. Using awake rabbits allowed us to compare directly the results in anesthesia-exposed animals versus unanesthetized controls. Our findings revealed significant learning impairment for the trace ECC paradigm in rabbits exposed to anesthesia during infancy, as well as significant functional changes in the cortex.

Disclosures: **D.P. Aksenov:** None. **M. Miller:** None. **L. Li:** None. **A. Wyrwicz:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH 5R37MH087027

Title: Dynamics of prefrontal, medial temporal lobe, and caudate networks in context-specific associations

Authors: *M. MOAZAMI GOUDARZI, S. L. BRINCAT, E. K. MILLER;
MIT, Cambridge, MA

Abstract: Flexible, context-specific retrieval and expression of learned information is a hallmark of intelligent behavior. Multiple brain regions—including prefrontal cortex (PFC), the medial temporal lobe (MTL), and the caudate nucleus (Cd)—have been implicated but neural information processing within and network-level interactions between these areas are not well understood. We recorded spiking and local field potentials (LFPs) from up to 104 electrodes in the PFC, MTL (hippocampus and perirhinal cortex), and the caudate tail of monkeys performing a context-specific association task. One of four object cues was shown in one of four locations. Half of the 16 resulting object/location pairs mandated a leftward saccadic response following a brief delay, while the other half mandated a rightward saccade. Thus, the correct behavioral response required the integration of object and its location context. In PFC and Cd, information about both object identity and its spatial context were encoded in both multiunit spiking rates and evoked potential amplitudes. At a slightly longer latency, both signals in these regions also reflected the saccade direction imposed by the object/location pair. In contrast, the MTL regions encoded object identity and location only in their LFPs, but not in spiking activity, while the saccade was reflected in both signals. As LFPs are thought to primarily reflect feedforward input, and spikes reflect outputs, the MTL may be integrating feedforward object and spatial information to compute the associated saccade. We also used representational similarity analysis to examine interactions between these regions. Analysis of band-limited LFP signals showed the strongest interactions in the beta band (~10-25 Hz) with peaks during the pre-cue and delay periods and a trough during the cue period itself. These beta-band interactions were broadly similar between all pairs of brain regions. In contrast, analysis of the raw LFP signals revealed a cue-period peak at approximately the same time as the beta-band trough. CA3-PFC and Cd-PFC interactions exhibited only this phasic peak, while CA1-PFC interactions were sustained though the entire delay period. These results indicate widespread network interactions with more focused sustained interactions between CA1 and PFC.

Disclosures: **M. Moazami Goudarzi:** None. **S.L. Brincat:** None. **E.K. Miller:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: 4R01MH065252

Title: Multiple tasks reveal the neural basis of implicit and explicit learning

Authors: *R. LOONIS[1,2], E. G. ANTZOULATOS[3], S. L. BRINCAT[1], E. K. MILLER[1];
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Abstract: Learning can be explicit (hippocampal-dependent, and conscious) or implicit (non-hippocampal dependent, and unconscious). We found neural evidence for both in a meta-analysis of six different monkeys performing three different learning tasks: (1) an object paired-associate matching task, (2) a category-saccade association task, and (3) a category matching task. All involved trial and error learning. Correct responses produced reward (positive feedback) and incorrect responses produced a visual signal and a time-out (negative feedback). Category-saccade learning is likely to be more implicit as it involved learning a motor response. The learning of category-saccade associations was primarily driven by reward, and monkeys reverted to chance performance following an incorrect response. This behavioral pattern suggests implicit learning, because amnesiacs who by definition employ implicit strategies learn better when positive feedback is emphasized. On the other hand, the paired-associate and category matching tasks are likely to be more explicit as they involved matching judgments. Learning the correct matches involved both positive and negative feedback, and behavior improved after both. The implicit (saccade) task and the explicit (matching) tasks had different patterns of within- and cross-area synchronizations during the feedback period. During category-saccade learning, there was theta band (2-7 Hz) synchrony on correct trials within and between prefrontal cortex and striatum. This weakened with learning and then, late in learning, appeared on incorrect trials. By contrast, during the matching tasks, there was beta band (10-25 Hz) synchrony on correct trials and that, in the category-matching task, also weakened with learning. Thus, theta and beta synchrony may help drive the plastic changes underlying implicit vs explicit learning, respectively.

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Poster

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Title: Training in a working memory task affects neuronal activity differentially along the anterior-posterior axis of the primate prefrontal cortex

Authors: *M. RILEY, X. QI, C. CONSTANTINIDIS;
Neurobio. and Anat., Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The prefrontal cortex (PFC) is a brain area critically involved in working memory (WM) and executive function. A functional gradient of specialization along the anterior-posterior axis of the PFC has been speculated, with posterior areas representing more faithfully the properties of stimuli, and anterior areas activated by more abstract operations, but little neurophysiological evidence exists to support it. To address this idea, we trained monkeys in a spatial WM task requiring subjects to determine if a stimulus was either in the same spatial location as a previously presented stimulus or not. We then examined the effects of training on 3 prefrontal regions: a posterior dorsal region, including area 8A, a mid-dorsal region including area 8B and area 9/46, and an anterior dorsal region, including area 9 and area 46 of Petrides and Pandya. A total of 669 neurons were recorded in 4 monkeys after training and were compared to 1046 neurons recorded while the same monkeys were exposed to these stimuli passively before training in any WM task. After training, a greater proportion of anterior-PFC neurons were activated by the stimuli during the delay period than before training (before 7%/after 36%, a 4.1 fold increase). This increase was less pronounced for posterior regions (posterior-PFC: before 18%/after 40%; mid-PFC: before 24%/after 36%). Among responsive neurons, a greater increase in firing rate to the stimulus was also seen in the anterior prefrontal cortex after training (before: 4.4/after: 9.3 spikes/s) compared to posterior (before: 7.6/after: 6.8 spikes/s) and mid-PFC (before: 4.6/after: 5.3 spikes/s). Similarly, mean stimulus selectivity among neurons active during the trial, quantified with a selectivity index as (Max-Min)/(Max+Min), was low in the anterior areas before training and increased appreciably following training, whereas it changed little or declined after training for the mid- and posterior-PFC regions (posterior-PFC, before: 0.78/after: 0.51; mid-PFC, before: 0.58/after: 0.55; anterior-PFC, before: 0.44/after: 0.64; 2-way ANOVA, $p<0.0001$, for interaction). The percentage of explained variance for the spatial location of stimuli exhibited a similar pattern (posterior-PFC, before: 25.5%/after: 11.0%; mid-PFC, before: 13.4%/after: 13.1%; anterior-PFC, before: 4.7%/after 17.0%; 2-way ANOVA, $p<0.0001$, for interaction). Our results provide neurophysiological evidence that anterior PFC neurons are more plastic with respect to a task being executed, and their functional properties change to a greater extent than posterior/middle PFC neurons, providing evidence of specialization along the anterior-posterior axis of the PFC.

Disclosures: **M. Riley:** None. **X. Qi:** None. **C. Constantinidis:** None.

Poster

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Tab Williams Endowment Fund

Title: Delay period activity in dorsolateral prefrontal cortex related to working memory capacity

Authors: *H. TANG, X. QI, M. R. RILEY, C. CONSTANTINIDIS;
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Abstract: The capacity of information that can be stored in human working memory is severely limited, typically to approximately 4 items. Studies in non-human primates have also obtained estimates of limited capacity. Little is known about how neural activity relates to working memory capacity and the nature of this severe limitation. To investigate this question, we trained one monkey to perform a spatial working memory task, requiring memory for the locations of multiple stimuli. The monkey viewed a sample display with 1 to 5 white squares presented for 0.5 s. The stimuli could appear at one of 24 locations arranged on a circle, at an eccentricity of 10 degrees of visual angle. After a delay period of 1 s, a second display appeared with the same number of stimuli and with all the items appearing at the same locations as in the sample or with one of the squares appearing at a different location. Two choice targets appeared subsequently, one of them green and the second blue. The monkey was required to saccade to the green target if the two stimulus displays were identical, and to the blue target if they were not. The monkey's behavioral performance was determined as a function of the number of stimuli. As expected, overall performance declined as the number of stimuli increased. The subject's estimated capacity in the task reached an asymptote at approximately K=3.5 items. After the monkey was trained in the task, we recorded from a total of 104 neurons in the dorsolateral prefrontal cortex. We focused particularly on neurons with significant selectivity for different stimulus locations (ANOVA, $p<0.05$). If prefrontal delay period activity represents the stimuli stored in memory, we reasoned, then as additional items are stored overall activity would be expected to increase across the population. When the capacity limit is exceeded, delay period activity representing some of the stimuli decays, and the items cannot be recalled at the end of the delay period. Addition of more stimuli should no longer produce increases in mean activity across the population. Indeed, we observed that delay period activity increased as a function of number of stimuli, for up to 3 items. For displays with 4 or more stimuli, which exceeded the subject's behavioral capacity, persistent activity decreased, a statistically significant difference across conditions (ANOVA, $p<0.01$). These results suggest that prefrontal delay period activity is

predictive of the subject's capacity limit. Our findings provide mechanistic insights on the working memory capacity limitation.

Disclosures: **H. Tang:** None. **X. Qi:** None. **M.R. Riley:** None. **C. Constantinidis:** None.

Poster

550. Executive Function: In Vivo Recording and Functional Study

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 550.09/KKK55

Topic: H.01. Animal Cognition and Behavior

Support: NIMH 4R01MH065252

Title: Category representations in prefrontal cortex on different levels of category abstractness

Authors: ***A. WUTZ**, J. E. ROY, E. K. MILLER;
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Abstract: The ability to group and organize patterns of sensory input into categories is a hallmark of cognition. Sometimes category members can look physically similar. Other times, however, categories are highly abstract and the individual exemplars can look quite different (e.g. "tools").
We compared category selectivity in the prefrontal cortex (PFC) of rhesus macaque monkeys at different levels of category abstractness using a dot-pattern categorization task (Posner & Keele, 1968). Abstractness of category exemplars was varied by the degree of spatial distortion of the dot pattern from the category prototype. Thus, low distortion exemplars look similar to members of the same category and different from the other category. Under high distortion, exemplars from the same category can look very different and thus require greater abstraction of the "essence" of the category prototype. Monkeys performed a delayed-match-to-category task that required judgments of whether two exemplars (sample – test) were from the same category. For each recording session, monkeys learned to distinguish between two novel categories. We recorded local field potentials (LFPs) from multi-electrode arrays in ventrolateral and dorsolateral PFC (VLPFC, DLPFC).
Behavioral results yielded the expected effects of better categorization with lower levels of distortion. Category selectivity was seen in the stimulus-evoked LFP signal in DLPFC and in beta frequency-band power in VLPFC. During sample exemplar presentation, category information in the evoked LFP was equally strong for high and low-level distortions in DLPFC. However, during the memory delay and subsequent category match judgment of the test stimulus, it showed less information about the sample category for more distorted exemplars.

Similarly, VLPFC beta power (25-30 Hz) during the delay period only showed category information for low-distortion exemplars. Multivariate pattern category decoding in DLPFC was above chance when trained on one distortion level and tested on the other. Thus, the PFC seemed to abstract the same essential category information across the different distortion levels. In sum, PFC activity could signal category membership at high levels of abstraction (distortion) but this selectivity was weaker, mirroring the monkey's greater difficulty for more abstract categorization.

Disclosures: **A. Wutz:** None. **J.E. Roy:** None. **E.K. Miller:** None.

Poster

550. Executive Function: In Vivo Recording and Functional Study

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 550.10/KKK56

Topic: H.01. Animal Cognition and Behavior

Support: CHARGE Foundation

Title: Investigating the role of CHD7 in glutamatergic synaptic development

Authors: ***A. P. ARANDA**, F. L. W. LIEBL, T. DELANEY;
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Abstract: The nervous system depends on the proper development and function of synapses. Glutamatergic synapses are essential for multiple processes including learning and memory. We have found that the chromodomain helicase DNA (CHD) binding protein, Kismet (Kis), regulates glutamate receptor localization and synaptic transmission at the *Drosophila* neuromuscular junction. One of the mammalian orthologs of *kis*, *CHD7*, is important for early neurodevelopmental events including migration of neural crest cells. Mutations in *CHD7* lead to CHARGE Syndrome, which is characterized by delayed neurodevelopment and sensory abnormalities. The synaptic role of CHD7, however, is largely unexplored. We are using the *Gal4-UAS* expression system to express a *Drosophila*-optimized *CHD7* in controls and *kis* mutant flies to assess the functional redundancy between Kis and CHD7.

Disclosures: **A.P. Aranda:** None. **F.L.W. Liebl:** None. **T. Delaney:** None.

Poster

550. Executive Function: In Vivo Recording and Functional Study

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH 4R01MH065252 (EKM)

Prop. 63 the Mental Health Services Act and the Behavioral Health Center of Excellence at UC Davis (EGA)

Title: Synchronous beta rhythms of frontoparietal networks support only behaviorally relevant representations

Authors: *E. G. ANTZOULATOS[1,2], E. K. MILLER[2];
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Abstract: Executive functions of the primate brain have been associated with distributed networks spanning several brain areas, including the prefrontal cortex (PFC) and the posterior parietal cortex (PPC). Both the PFC and PPC have been previously shown to play roles in rule-based categorization. Although category-selective spiking activity in these areas has been established, the frequency-dependent dynamic interactions of frontoparietal networks are largely unexplored. We trained monkeys to perform a delayed-match-to-spatial-category task while recording spikes and local field potentials (LFPs) from the PFC and PPC with multiple electrodes. We found category selective beta- and delta-band synchrony between and within the areas; different pairs of sites showed greater synchrony for one or the other category. However, in addition to the categories, delta synchrony and spiking activity also reflected irrelevant stimulus dimensions. By contrast, beta synchrony only conveyed information about the task-relevant categories. Further, the spikes of category-selective PFC neurons were synchronized with PPC beta oscillations. PFC neurons that carried irrelevant information were not. These results suggest that long-range beta-band synchrony could act as a filter that only supports neural representations of the variables relevant to the task at hand.

Disclosures: E.G. Antzoulatos: None. **E.K. Miller:** None.

Poster

550. Executive Function: In Vivo Recording and Functional Study

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Program#/Poster#: 550.12/KKK58

Topic: H.01. Animal Cognition and Behavior

Support: NIH grant R01 EY016773

Tab Williams Endowment Fund

Title: Influence of prefrontal and posterior parietal delay period activity on the accuracy of spatial working memory

Authors: *X. QI[1], X. ZHOU[2], S. LI[1], C. CONSTANTINIDIS, 27103[1];
[1]Wake Forest Univ. Sch. of Med., Winston Salem, NC; [2]Stanford Univ., Stanford, CA

Abstract: The dorsolateral prefrontal (dlPFC) and posterior parietal cortex (PPC) are involved in cognitive operations such as working memory and executive function. Only subtle differences in firing rate have been observed between neurons recorded in the two areas, across various cognitive tasks. Here we addressed differences between the two areas on how their activity influences working memory performance on a single trial basis. We trained two monkeys in the Oculomotor Delayed Response task which requires the monkey to saccade to the remembered location of a stimulus, after a delay period. The accuracy of working memory recall was evaluated based on the distance of the saccadic end point from the true location of the stimulus. Neurophysiological recordings were performed in areas 8a and 46 of the dlPFC and 7a and LIP of the PPC. We selected neurons with significantly elevated activity and significantly different responses to different stimulus locations (ANOVA, $p<0.05$) during the delay period. We collected 170 such neurons from PFC and 63 from PPC. We first compared the tuning strength of neurons in each area. Neurons in dlPFC showed a significantly higher tuning strength, indicating stronger selectivity for the location of the stimuli during the delay period, compared to the PPC (t-test, $p<0.05$). We then examined the correlation between delay period activity and the accuracy of the saccadic end point across trials. Prefrontal neurons showed significant correlations between firing rate and saccadic deviation for stimulus locations appearing at the flank of their tuning curve, but not for locations at the tail of the tuning curve. Parietal neurons also showed a correlation during the delay period for flank locations but also for tail locations, particularly later in the delay period. This correlation rose with increasing tuning strength for dlPFC and PPC. Tuning curves constructed based on the firing rate recorded in trials with saccadic endpoints deviating clockwise or counterclockwise from the true stimulus location exhibited a slight difference, or tuning bias, for neurons in both areas. The tuning bias was higher overall for dlPFC neurons than PPC neurons. The results indicate that the activity of neurons

both in prefrontal and in posterior parietal cortex influences the accuracy of spatial working memory recall, but that systematic differences exist between areas.

Disclosures: **X. Qi:** None. **X. Zhou:** None. **S. Li:** None. **C. Constantinidis:** None.

Poster

550. Executive Function: In Vivo Recording and Functional Study

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Program#/Poster#: 550.13/KKK59

Topic: H.01. Animal Cognition and Behavior

Support: NIMH 5R01MH091174

NIMH 5R37MH087027

Title: Working memory load may modulate neuronal coupling

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[1]MIT, Cambridge, MA; [2]Princeton Neurosci. Inst. and Dept. of Psychology, Princeton Univ., Princeton, NJ

Abstract: There is a severe limitation in the number of objects that can be held in working memory. However, the neurophysiological origins of cognitive capacity remain unknown. In this work, we focus on the biophysical correlates of the bandwidth limit of conscious thought and ask whether this might be explained by differences in load-specific neuronal coupling. We develop a theoretical model based on Predictive Coding and use it to analyze local field potentials (LFPs) recorded with multiple electrodes in prefrontal cortex (PFC), frontal eye fields (FEF) and lateral intraparietal area (LIP), while monkeys performed a multiple object working memory task (Buschman et al., 2011). In previous work (Kornblith et al.,2015), we calculated the power of oscillatory responses recorded from this task and found that it depends crucially upon the number of maintained objects. Interestingly, power increases with memory load beyond behavioral capacity; however, it is unclear how increasing load affects neuronal coupling and whether the capacity limit can be explained by changes in the extrinsic and intrinsic connectivity in the PFC-FEF-LIP network. Our model attempts to address these questions based on Predictive Coding and the data from Buschman et al. (2011). This is a theory of brain function which suggests that brain regions are hierarchically organized and interact in both feedforward and feedback directions. It also presumes that the activity of deep and superficial pyramidal cell populations reflects the processing and maintenance of visual stimuli. Motivated by these considerations, our model predicts that top down signals maintaining sensory predictions would result in increased

feedback connectivity from PFC to FEF and LIP and an increase in the gain of deep pyramidal cell populations with varying memory load. Here we test these predictions and investigate whether the memory encoding of visual objects can be explained by load-specific differences in extrinsic and intrinsic cortical connectivity. In brief, our study provides new insights into the neuronal underpinnings of behavioral capacity and how effective connectivity in a distributed working memory network is affected by a different number of remembered stimuli.

Disclosures: D. Pinotsis: None. **T.J. Buschman:** None. **E.K. Miller:** None.

Poster

550. Executive Function: In Vivo Recording and Functional Study

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 550.14/DP08 (Dynamic Poster)

Topic: H.01. Animal Cognition and Behavior

Title: Effects of D-serine on visual working memory in macaque monkey

Authors: *M. KUSI, J. MANJUNATH, C. CRANDELL, V. BARRETTE, M. PARÉ;
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Abstract: Working memory is a limited-capacity cognitive process that retains information for short periods of time to guide thoughts and behavior. A neural correlate of working memory has been postulated in the persistent activity of neurons in the cerebral cortex during the memory retention intervals of various behavioural tasks. Modeling and behavioral work suggests that this persistent activity depend on NMDA receptor (NMDAR) activation. Indeed, we have previously shown that the NMDAR antagonist ketamine induces deficits in visual working memory (VWM) that are dependent on both dose and memory load in monkeys. NMDAR hypofunction is now a dominant hypothesis to explain cognitive symptoms in schizophrenia, and the NMDAR co-agonist site has become a target for therapy tested in clinical trials. Here, we tested the effects of orally administered doses of the NMDAR co-agonist D-serine (acute 1-1000 mg/kg; sub-chronic 100mg/kg/day-6 weeks) on the VWM ability of three adult female rhesus macaques. We also tested the effects of D-serine (acute 100 mg/kg) on rescuing impaired VWM ability following ketamine (acute 0.5 mg/kg). Animals performed a sequential comparison task which required them to identify the location of a color change within an array of 2-5 colored stimuli following a retention interval (Heyselaar et al., 2011). We predicted that D-serine would improve performance on the VWM task and that the effects of D-serine would scale with memory load as well as being dose-dependent for acute treatment and time-dependent for sub-chronic treatment. Contrary to our predictions, we found that none of the tested doses of D-serine had an effect on the accuracy or latency of task responses at any memory load. We also found that D-serine did

not significantly improve task accuracy when ketamine was administered prior to D-serine administration. These findings suggest that targeting the NMDAR co-agonist site has a limited role in benefiting VWM.

Disclosures: **M. Kusi:** None. **J. Manjunath:** None. **C. Crandell:** None. **V. Barrette:** None. **M. Paré:** None.

Poster

550. Executive Function: In Vivo Recording and Functional Study

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Program#/Poster#: 550.15/KKK60

Topic: H.01. Animal Cognition and Behavior

Support: NIH DC0485

Schmitt Integrative Neuroscience (BP)

Center for Visual Science

Title: Activity of medial prefrontal and striatal neurons in primates while remembering faces and vocalizations.

Authors: *B. PLAKKE, L. M. ROMANSKI;
Dept. of Neurobio. & Anat., Univ. of Rochester Sch. of Med. and Dent., Rochester, NY

Abstract: Our previous work has shown that ventrolateral prefrontal cortex (VLPFC) integrates face and vocal information and is necessary for audiovisual working memory. However, visual working memory relies on many cortical and subcortical regions including the medial prefrontal cortex (mPFC) and dorsal striatum. Moreover, mPFC is involved in conflict detection, audio-vocal control and receives strong inputs from higher order auditory processing regions within the temporal lobe. There are few studies which have examined the roles of these regions in audiovisual processing and memory. Here, we recorded from mPFC and striatal neurons while macaques performed an audiovisual nonmatch-to-sample task. Subjects attended an audiovisual movie clip of a face-vocalization as the Sample and detected the occurrence of a Nonmatch (when the face or vocalization differed from the Sample movie). Neurons within the mPFC and striatum were task related and were active for key periods of the task including the sample, the delay, the match, and the nonmatch epochs. In contrast to previous findings from VLPFC, both mPFC and striatal neurons were more active during decision-related epochs of the task, such as detecting the Nonmatch or suppressing a response during a Match presentation than during the Sample or Delay. These decision related units were more commonly found in mPFC than in the

striatum. This data suggests mPFC and the striatum are part of a neuronal circuit underlying audiovisual working memory in the primate brain.

Disclosures: **B. Plakke:** None. **L.M. Romanski:** None.

Poster

550. Executive Function: In Vivo Recording and Functional Study

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Program#/Poster#: 550.16/KKK61

Topic: H.01. Animal Cognition and Behavior

Support: NIMH 5R01MH065252

NSF DMS-1042134

NIH 1R21MH108981

Title: Low-beta oscillations turn up the gain during category judgments

Authors: *D. A. STANLEY[1], J. E. ROY[2,3], N. J. KOPELL[1], E. K. MILLER[2,3];
[1]DEPARTMENT OF MATHEMATICS AND STATISTICS CAS/BU, Boston Univ., Boston, MA; [2]The Picower Inst. for Learning and Memory, [3]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Synchrony between local field potential (LFP) rhythms is thought to boost the signal of attended sensory inputs. Surely, other cognitive functions could benefit from such gain control. One is categorization where decisions can be difficult if categories differ in subtle ways. Monkeys were trained to flexibly categorize smoothly varying morphed stimuli, using orthogonal boundaries to carve up the same stimulus space. First, we found evidence for category-specific patterns of low-beta (16-20 Hz) synchrony in the prefrontal cortex. This synchrony was stronger when a given category scheme was relevant. Second, during sample presentation, we observed an overall increase in low-beta LFP synchrony for stimuli that were near the currently relevant category boundary and thus more difficult to categorize. We could identify single unit correlates of category sensitivity but not of the boundary effect. We also found that the category response showed up in partial FFC measurements, but the boundary response was almost completely abolished. Based on this, we propose that the category and boundary responses represent two distinct low-beta oscillations that work together; one local and the other system-wide. Increases in low-beta synchrony may then reflect recruitment of additional cortical resources for categorizing challenging stimuli, perhaps serving as a form of gain control.

Disclosures: **D.A. Stanley:** None. **J.E. Roy:** None. **N.J. Kopell:** None. **E.K. Miller:** None.

Poster

550. Executive Function: In Vivo Recording and Functional Study

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 550.17/KKK62

Topic: H.01. Animal Cognition and Behavior

Title: The use of zebrafish to identify genes affecting working memory and age-related cognitive decline.

Authors: *A. J. BROCK, A. SUDWARTS, C. H. BRENNAN;
SBCS, Queen Mary, Univ. of London, London, United Kingdom

Abstract: Aim
To identify genes influencing cognitive performance (memory and learning, impulse control) using zebrafish as a model system.
Method
GBT Screen: We screened lines of mutant zebrafish at 3 months for performance in behavioral tasks that assess aspects of cognition. So far we have screened over 50 gene-break transposon (GBT) lines (n=4 for each line) as well as wild-type (n=20) in a 5-choice serial reaction time task (5-CSRTT) starting at the 3-month stage. The 5-CSRTT system is fully automated as designed by Parker et al. (2012).
Development of MTS system: In collaboration with Bill Budenburg (Zantiks Ltd, Cambridge, UK) we have developed an automated, scalable system for measuring a number of behaviors including matching-to-sample (MTS), a measure of memory. The system is designed along the lines of a 'Skinner Box' for zebrafish can currently be used to measure 2-5 choice discrimination as well as MTS. Stimuli are presented using a computer screen positioned beneath the holding tank.
Results
GBT Screen: We have identified several lines of interest that show significantly altered performance from controls. When measuring the mean number of correct responses in the task, 2 lines ($p=0.03$ & $p=0.001$) clustered to the right of the distribution. There were 2 lines ($p=0.02$ & $p=0.05$) showing faster response when assaying latency to approach the stimuli. When using trials to steady state as a measure of learning rate 2 line ($p=0.03$ & $p=0.05$) show faster learning rates and one line ($p=0.01$) slower. Work is ongoing to rescreen the siblings from these families and characterize the mutations involved.
Automated Operant system: Using this system, we have trained adult 4 month (at start)

zebrafish to perform 3-choice discriminations to over 80% accuracy within 3 weeks, 5-choice discriminations to 70% accuracy within 5 weeks and 3-choice matching-to-sample to over 60% accuracy within 8 weeks.

Discussion Recently a number of high profile publications have suggested that zebrafish can also be used to explore the genetics and aetiology of behavior including complex behavioral phenotypes associated with psychiatric disease. However, progress has been limited. This is partly due to the lack of an automated system that can be used for analysis of cognitive behaviors in large-scale genetic or pharmaceutical screens.

We demonstrate that in our old 5-CSRTT system using a population screen approach, differing lines can be shown to cluster on either side of a distribution curve, demonstrating the ability to pick out genes affecting aspects of cognition. By developing the new operant system which is capable of measuring MTS we will be able to screen current GBT lines for their performance in this task.

Disclosures: **A.J. Brock:** None. **A. Sudwarts:** None. **C.H. Brennan:** None.

Poster

551. Learning and Memory: Amygdala and Hippocampal Circuits

Location: Halls B-H

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant 5R01MH102595-03

Training Grant 1T32MH106454-01

Title: Spaced context exposure enhances context fear memory and hippocampal context coding

Authors: *A. F. LACAGNINA[1], C. A. DENNY[2], M. R. DREW[1];
[1]Dept. of Neurosci., The Univ. of Texas at Austin, Austin, TX; [2]Dept. of Psychiatry, Columbia Univ., New York City, NY

Abstract: Spaced training is superior to massed training in many learning paradigms. Although the spacing effect has been studied in *in vitro* hippocampal preparations, its mechanisms *in vivo* are not well understood. We examined the effects of spaced versus massed context exposure in one-trial contextual fear conditioning (CFC), a hippocampus-dependent learning paradigm that depends on two learning processes: 1) forming a mental representation of the context and 2) associating that representation with an aversive stimulus. Theories of CFC posit that such mental representations can be acquired during passive exploration of an environment and are essential

for conditioning but do not make strong predictions about how varying the interval between context preexposure and conditioning will affect learning. Because the two learning processes can be separated in time, we predicted that introducing a delay between context preexposure and conditioning would strengthen CFC. We found that context preexposure 72 h or 24 h before single-shock CFC produced stronger conditioned fear than did preexposure 1 min before or no preexposure. A series of behavioral experiments revealed the effect of spaced context exposure was not explained by (1) a change in the susceptibility to extinction, (2) artifacts related to experiencing a novel context prior to conditioning, or (3) modulation of memory retrieval by primacy or recency. The results instead suggest that spaced context exposure strengthened context fear memory. To understand the neural activity patterns corresponding to the stronger conditioned fear following spaced preexposure, we used ArcCreER^{T2} x ChR2-eYFP mice to indelibly tag neurons activated during CFC. Mice were preexposed to the conditioning context 72 h or 1 min prior to one-trial CFC. Mice received an injection of 4-OHT prior to CFC acquisition, thereby labeling neurons active during CFC acquisition with eYFP. Five days later, mice were re-exposed to the shock-paired context and euthanized 90 min later. We compared neurons active during acquisition (eYFP+) to those activated by memory retrieval (cFos+). Mice that received preexposure 72 h prior to conditioning displayed an increased percentage of reactivated (cFos+/eYFP+) cells in CA3 compared to mice receiving preexposure 1 min prior. Moreover, the percentage of reactivated cells correlated with fear expression. In summary, spaced context exposure enhances CFC acquisition and increases the reactivation of CA3 neural ensembles associated with acquisition. These data demonstrate that trial spacing can modulate CFC and identify a neural correlate of the spacing effect in hippocampal CA3 related to memory strength.

Disclosures: **A.F. Lacagnina:** None. **C.A. Denny:** None. **M.R. Drew:** None.

Poster

551. Learning and Memory: Amygdala and Hippocampal Circuits

Location: Halls B-H

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Program#/Poster#: 551.02/KKK64

Topic: G.01. Appetitive and Aversive Learning

Support: ANR PTSDMEMO

Title: Switching from normal to PTSD-related fear memory is associated with epigenetic modifications in the hippocampus-amygdalar circuit

Authors: ***C. BOUARAB**[1,2], M. MENNESSON[3], C. GUETTE[1], D. N. ABROUS[1], A. MARIGHETTO[1], M. KOEHL[1,2], N. MONS[2,4], A. DESMEDT[1,2];

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Abstract: Posttraumatic stress disorder (PTSD) is characterized both by hypermnesia for simple salient trauma-related stimuli and amnesia for peri-traumatic contextual cues. In human, this disorder is associated with hippocampal hypofunction but amygdalar hyperfunction, which may contribute to such paradoxical memory impairment. We recently showed that PTSD-related memory could be mimicked in mice in which the erroneous selection of a salient tone stimulus instead of the context as predictor of the threat is observed in association with an altered pattern of neural activation within the hippocampal-amygdalar network. On this basis, the molecular changes underlying this system dysfunction can now be investigated.
Here we show that, compared to normal fear memory, PTSD-like memory is associated with a switch from H3K9 hyperacetylation to H3K9 hypermethylation in hippocampal CA1 as well as a significant reduction of H3K27 trimethylation (non-permissive) resulting to an increase of transcription in the amygdala. In addition, we show that prenatally stressed mice exposed to a stressful event in adulthood develop a PTSD-like memory and display a similar histone H3 dysregulation pattern. Altogether, these results indicate that an altered histone H3 acetylation/methylation balance within the hippocampal-amygdalar network may contribute to the switch from normal to pathological memory.

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Poster

551. Learning and Memory: Amygdala and Hippocampal Circuits

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Topic: G.01. Appetitive and Aversive Learning

Support: Kaken-hi (15H05569)

Kaken-hi (15H01417)

Title: Social defeat-induced changes in network oscillations in the rat hippocampus

Authors: ***R. NAKAYAMA**, S. OKADA, T. SASAKI, Y. IKEGAYA;
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Abstract: Chronic social defeat is a widely used animal model with high etiological and discriminative validity that evokes profound behavioral phenotypes, including increased anxiety and depression-like syndromes. Many studies have revealed the effects of social defeat stress on molecular and cellular functions of various organs. In this study, we examined how social defeat stress alters physiological activity in freely moving rats. To this end, we developed a new recording technique that can simultaneously monitor ongoing bioelectrical signals from the brain and peripheral organs. The recording system integrates local field potential signals from the cortex, heartbeat signals (electrocardiograms), and skeletal muscle signals (electromyograms) into an electrical interface board that is mounted on an animal's head. We applied this method to intruder rats that were subject to a social defeat paradigm using an aggressive resident rat and analyzed their cortical activity patterns by comparing a variety of physiological body states. After receiving a physical attack from the resident rat, the heart rate of the intruder animals was transiently increased and then decreased when they started to show freezing behavior, a sign of subordination. During the freezing period, hippocampal ripple events, which represent synchronous firing of hippocampal pyramidal cells, were prominently abolished. This result is in contrast to the general notion that hippocampal ripples preferentially occur during rest/immobility periods and suggests that the social defeat-induced freezing behavior does not include ripple-dependent memory processing such as consolidation and retrieval. During periods without freezing, the frequency of hippocampal ripples was negatively correlated with the heart rate. We will further analyze whether other types of cortical oscillatory activity is related to body states. The evidence will advance the understanding of the neurophysiological correlate of mind-body associations during mental stress exposure.

Disclosures: **R. Nakayama:** None. **S. Okada:** None. **T. Sasaki:** None. **Y. Ikegaya:** None.

Poster

551. Learning and Memory: Amygdala and Hippocampal Circuits

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Topic: G.01. Appetitive and Aversive Learning

Support: NIMH R01 MH078064

Title: Hippocampal-retrosplenial connectivity is necessary for memory retrieval after contextual fear conditioning

Authors: *K. A. CORCORAN, B. J. FRICK, A. L. GUEDEA, J. RADULOVIC;
Psychiatry & Behavioral Sci., Northwestern Univ., Chicago, IL

Abstract: Memory retrieval requires activity in a number of brain regions. We have previously demonstrated essential roles for both dorsal hippocampus (DH) and retrosplenial cortex (RSC) in contextual fear conditioning, though little is known about how these two structures interact to mediate the retrieval of conditioning memories. Here, we used an adeno-associated viral vector to deliver inhibitory DREADDs to specific excitatory projections from DH to RSC in mice. After contextual fear conditioning, mice were tested for memory retrieval after intra-RSC infusions of aCSF and clozapine-n-oxide (CNO), the synthetic ligand that activates the DREADDs in order to inhibit neural activity. By delivering the DREADDs to DH and CNO to RSC, we were able to specifically inhibit activity in hippocampal axon terminals in RSC and disrupt hippocampus-retrosplenial communication. CNO reduced freezing to the conditioning context without causing hyperactivity, suggesting that excitatory DH-RSC projections mediate retrieval of contextual fear conditioning memory. In a second experiment, DREADDs were again delivered to DH, and insulated silver wires were implanted in DH and RSC. We recorded local field potentials (LFPs) and measured intra-regional power and inter-regional coherence of oscillatory activity in the delta (1-4Hz), theta (4-12Hz) and gamma (30-80Hz) frequency bands from both regions during novel context processing and memory retrieval. By combining targeted inhibition of specific excitatory inputs and in vivo electrophysiology, we can begin to understand the nature of the inter-regional structural and functional connectivity that underlie memory retrieval.

Disclosures: K.A. Corcoran: None. **B.J. Frick:** None. **A.L. Guedea:** None. **J. Radulovic:** None.

Poster

551. Learning and Memory: Amygdala and Hippocampal Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 551.05/KKK67

Topic: G.01. Appetitive and Aversive Learning

Support: MH078064

Title: Sexually dimorphic effects of state-dependent memory on social and affective behaviors

Authors: *M. MEYER, G. HAST, J. RADULOVIC;
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Abstract: Patients suffering from stress-related disorders, in particular post-traumatic stress disorder (PTSD), commonly have dissociative symptoms related to their inability to properly recall the traumatic event. These trauma-related memories are not forgotten, but only

inaccessible under normal conditions. Instead, these memories are state-dependent, such that they can be retrieved when patients are in a similar psychological or physiological state as when the trauma occurred. Dissociative amnesia is highly correlated with deficits of social functioning, including abnormal social cognition and inability to maintain interpersonal relationships. Dissociative amnesia occurs at similar rates in males and females, however, it is well established that males and females cope with traumatic events differently, with a female bias towards affective PTSD symptoms. We therefore investigated phenotypic sexual dimorphism and circuit mechanisms of social and affective behaviors in a mouse model of state-dependent memory, including sociability, social recognition, and light-dark emergence. We demonstrated that state-dependent contextual fear conditioning mediated by pharmacological excitation of extrasynaptic gamma-aminobutyric acid receptors type A ($GABA_AR$) impairs sociability, successfully recapitulating the pairing of dissociative symptoms with social deficits observed in humans. Identifying the effects of state-dependent memory on social and affective behaviors is important for understanding the role of inaccessible stress-related information on behaviors executed in alternative brain states. Theme G: Motivation and Emotion G.01. Appetitive and Aversive Learning G.01.g. Fear and aversive learning and memory: Hippocampal related circuits G.06. Post-traumatic Stress Disorder G.06.b. preclinical models Keywords: $GABA_A$ Receptor Hippocampus Context

Disclosures: **M. Meyer:** None. **G. Hast:** None. **J. Radulovic:** None.

Poster

551. Learning and Memory: Amygdala and Hippocampal Circuits

Location: Halls B-H

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Program#/Poster#: 551.06/KKK68

Topic: G.01. Appetitive and Aversive Learning

Support: MH097860

Title: PACAP alters fear-related behavior and increases activity-regulated cytoskeleton-associated protein (Arc) expression in the central nucleus of the amygdala after fear conditioning in rats

Authors: *E. G. MELONI, K. T. KAYE, W. A. CARLEZON, Jr;
Psychiatry, McLean Hosp., Belmont, MA

Abstract: Pituitary adenylate cyclase-activating polypeptide (PACAP) is a highly conserved neuropeptide, with shared amino acid sequence homology among rats, mice, and humans. Alterations in the PACAP system are associated with stress and may play a role in psychiatric

conditions such as post-traumatic stress disorder (PTSD). We have previously shown that PACAP modulates learning and memory and induces both amnestic (early) and hypermnestic (late) behavioral phenotypes in animals treated with PACAP prior to fear conditioning (Meloni et al., 2015). In those studies, immunohistochemistry for the immediate early gene c-Fos revealed PACAP-dependent effects in the central nucleus of the amygdala (CeA), but not basolateral amygdala (BLA) or dorsal hippocampus (DH), brain areas implicated in consolidation of fear learning. Here, we examined PACAP's effects on synaptic plasticity in these brain areas by measuring changes in activity-regulated cytoskeleton-associated protein (Arc) expression, a biomarker of synaptic activity that could uncover plasticity-dependent effects not revealed by c-Fos in earlier studies. Male Sprague-Dawley rats were implanted with intracerebroventricular (ICV) cannula for infusion of either vehicle (VEH) or PACAP-38 (1.5 ug) followed 30 min later by fear conditioning; rats received two pairings of a 30 s, 75 dB tone co-terminating with a 0.6 mA footshock. Rats were perfused 1 hour after conditioning, and brain sections were processed for Arc immunoreactivity. There was a highly significant increase in Arc-labeled neurons in the CeA but not the bed nucleus of the stria terminalis (BNST) or DH (dentate gyrus) in PACAP-treated animals. In the CeA, double labeling immunohistochemistry indicated no co-localization between Arc and corticotropin releasing factor (CRF)-containing neurons in PACAP-treated animals. Arc staining in the BLA or dendritic fields of the CA1-CA3 of the DH were not significantly different between VEH and PACAP-treated rats. These results indicate that the CeA is a primary brain area involved in PACAP's modulation of neuronal plasticity induced during fear conditioning. Because the CeA receives heavy PACAPergic innervation from the brainstem, stress-induced elevations in this neuropeptide - as is seen in some patients with PTSD (Ressler et al., 2011) - may facilitate consolidation of emotional memories through Arc-dependent pathways. Hence, understanding the neurobiology of PACAP systems may help reveal how stressful experiences such as exposure to trauma promote psychiatric illness, and facilitate the development of more effective strategies to treat or prevent stress-related disorders.

Disclosures: **E.G. Meloni:** None. **K.T. Kaye:** None. **W.A. Carlezon:** None.

Poster

551. Learning and Memory: Amygdala and Hippocampal Circuits

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Title: p38 mitogen-activated protein kinase activation in amygdala mediates κ opioid receptor agonist U50,488H-induced conditioned place aversion.

Authors: *J.-G. LIU, G. ZAN;
Shanghai Inst. Materia Medica, Shanghai, China

Abstract: κ Opioid receptor agonists produce aversive effects in rodents, however the underlying mechanisms remain unclear. Activation of p38 mitogen-activated protein kinase (MAPK) has been discovered to play a critical role in the modulation of affective behaviors. The present study was undertaken to detect the possible involvement of p38 MAPK in the aversive effects induced by κ opioid receptor activation. We found that the κ opioid receptor agonist trans-(±)-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl] benzenacetamide methanesulfonate salt (U50,488H) produced significant place aversion in mice as measured by the conditioned place preference procedure, accompanied with significant p38 MAPK activation in the amygdala, but not in the nucleus accumbens and hippocampus. Stereotaxic microinjection of the p38 MAPK inhibitor 4-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)-5-(4-pyridy-l)-1H-imidazole (SB203580) into amygdala significantly inhibited p38 MAPK activation and completely blocked the conditioned place aversion in mice. Thus, these results suggested that activation of p38 MAPK in the amygdala was required to mediate κ opioid receptor-induced aversive behavior.

Disclosures: J. Liu: None. **G. Zan:** None.

Poster

551. Learning and Memory: Amygdala and Hippocampal Circuits

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant NS034007

NIH Grant NS047384

Title: Inducible protein synthesis inhibition in the amygdala.

Authors: *P. M. HERRERO-VIDAL[1], P. SHRESTHA[1], P. AYATA[2], A. GASTONE[1], N. HEINTZ[2], E. KLANN[1];
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Abstract: Extensive studies support the role of the amygdala as a major hub for integration, processing and consolidation of auditory fear conditioned responses. Hence, this brain region has been widely used for studying learning and memory processes at a molecular and cellular level. Although excitatory neurons of the lateral amygdala are thought to be the cellular substrate for fear memory formation, new evidence supports the importance of different types of interneurons in this process for which *de novo* protein synthesis appears to be necessary. Prior research using pharmacological protein synthesis inhibitors, such as cyclohexamide or anisomycin, have indicated that translation is required for long term fear memory formation. However, there is still considerable controversy regarding the relevance of these findings given the possibility that the observed results may be due to drug secondary effects. Here, we introduce a novel method to block general translation in an inducible and reversible manner as an alternative approach to study the role of newly synthesized proteins in this process. Initiation is a tightly regulated step in translation and depends on eukaryotic initiation factor 2α (eIF2α). In response to cellular stress, eIF2α is phosphorylated at the amino acid residue Serine 51 and results in inhibition of general protein synthesis. Making use of this molecular mechanism, we have modified the eIF2α kinase domain of PKR to be both drug-inducible and cell-type specific. First, we tested the system both *ex vivo* and *in vivo* in a Nestin-cre pan neuronal mouse line crossed to our inducible iPKR line using the drug, Asunaprevir, and obtained a significant and reversible translation block. We next confirmed our results by shutting down the protein synthesis wave following fear conditioning in amygdala and blocking the formation of the auditory fear memory. Administration of the drug resulted in reversible block of cued freezing response after drug administration. Finally, we immunohistochemically observed the activation of protein signaling cascades in the major interneuron cell types in the amygdala, Parvalbumin, Somatostatin and Protein Kinase C- delta expressing cells. The use of our cell-type specific pharmacogenetic system selectively expressed could therefore be used to ascertain the differential role of *de novo* protein synthesis in inhibitory neuron populations for the modulation of the cued fear memory.

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Poster

551. Learning and Memory: Amygdala and Hippocampal Circuits

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Program#/Poster#: 551.09/LLL1

Topic: G.01. Appetitive and Aversive Learning

Support: NCN Grant G2085

Title: c-Fos in the central amygdala in appetitively and aversively motivated learning

Authors: *T. LEBITKO[1], H. MADEJ[1], T. JAWORSKI[1], A. SUSKA[1], K. KONDRAKIEWICZ[2], E. KNAPSKA[2], L. KACZMAREK[1];
[1]Dept. of Mol. and Cell. Neurobio., [2]Dept. of Neurophysiol., Nencki Inst., Warsaw, Poland

Abstract: The central nucleus of the amygdala (CeA) is well known for its involvement in aversively motivated learning, e.g. in fear conditioning. However, neurons in the CeA are also essential for reward learning. Before, we reported that matrix metalloproteinase-9 (MMP-9, extracellularly operating enzyme) in the CeA is crucial for appetitive, but not for aversive, learning. Since in activated neurons MMP-9 is c-Fos/AP-1 regulated at the transcriptional level, we hypothesized that appetitively motivated learning depends also on learning-driven c-Fos expression in the CeA. To test this hypothesis we injected CeA with a short-hairpin (sh) RNA in a lentiviral vector. Blocking *c-fos* expression resulted in impairment of appetitively but not aversively motivated discrimination learning and significantly reduced motivation to seek for a reward. To further characterize c-Fos dependent motivational changes we optogenetically stimulated CeA neurons involved in reward learning. Animals were injected with genetic construct, in which channelrhodopsin is placed under the control of a *c-fos* promoter and trained in the operant conditioning chamber to associate an auditory stimulus with contiguous food reinforcement. Learning was defined as an ability to modify the bar-pressing responses during exposure to the signaling stimulus. Subsequent activation of the CeA neurons during the signaling stimulus presentation amplified incentive motivation for food reward reflected by an increase in the number of bar-pressing responses. Such stimulation did not change behavioral responses in the absence of a signaling stimulus. Taken together, the results show that c-Fos expression in the CeA is necessary for learning motivated by alimentary rewards. In particular, *c-fos*-expressing neurons modulate incentive motivation to pursue an associated reward.

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Poster

551. Learning and Memory: Amygdala and Hippocampal Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 551.10/LLL2

Topic: G.01. Appetitive and Aversive Learning

Title: Neural ensemble coding of nociceptive information in the amygdala drives innate pain affective behavior

Authors: *G. F. CORDER, B. AHANONU, B. GREWE, C. SOTOUDEH, M. SCHNITZER, G. SCHERRER;
Stanford Univ., Palo Alto, CA

Abstract: Pain is a cohesive multidimensional experience arising from sensory and emotional processes. Qualitative categorization of nociceptive sensory information as 'unpleasant' is essential for protective learning, behavior selection, and survival. Injury-induced plasticity within emotional valence circuits, such as the basolateral amygdala (BLA), may lead to a miscoding of sensory information concomitant with the emergence of chronic pain. To determine how the BLA encodes nociceptive information and how this activity changes during the development of chronic neuropathic pain, we manipulated nociresponsive BLA neural ensembles and followed their dynamics for multiple weeks after the induction of a peripheral nerve injury. To do this, we selectively expressed the inhibitory DREADD(hM4) receptor in a functionally-defined population of BLA nociresponsive neurons. Chemogenetic manipulation of the BLA nociceptive network in mice with nerve injury did not alter reflexive pain behavior. Strikingly, affective pain behaviors following noxious stimulation, such as attending to the injured paw and escape-avoidance, were dramatically reduced. To further characterize how BLA ensemble dynamics represent noxious stimuli before and after nerve injury, we conducted long-term *in vivo* Ca^{2+} imaging of $CaMKII^{+}$ BLA neurons using the miniature integrated fluorescence microscope and the Ca^{2+} indicator GCaMP6m. Prior to nerve injury, multidimensional and population vector analysis of sensory-evoked Ca^{2+} transients revealed that the BLA uniquely represents noxious and innocuous stimuli (*e.g.* sharp pin *vs*. light touch), as well as distinct sensory modalities (*e.g.* mechanical *vs*. thermal). After the establishment of neuropathic pain, the neural ensemble representations of prior innocuous and noxious stimuli became more similar. Overall, we have identified unique network representations of noxious information in the BLA; these representations facilitate the innate, negative affective qualities of the pain experience and drive appropriate motivated behaviors. Further, our findings demonstrate how BLA ensemble activity evolves under chronic pain states to assign negative valence to previously innocuous stimuli, possibly contributing to pathological sensory dysfunctions such as allodynia, and to psychological co-morbidities such as depression.

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Poster

552. Working Memory: Hippocampus and Cortex

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 552.01/LLL3

Topic: H.01. Animal Cognition and Behavior

Title: Functional effects of age on NR2A and NR2B containing NMDA receptors in interneurons and pyramidal cells of the rat medial prefrontal cortex

Authors: *K. B. KELLY[1], J. L. BIZON[2], C. J. FRAZIER[1];
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Abstract: Biochemical studies from our group and others have shown that NMDA receptor subunit expression is decreased in the aged prefrontal cortex and suggested these changes are associated with specific impairments in cognitive function. This study was designed to carefully evaluate age related changes in NMDA receptor mediated currents in both interneurons and pyramidal cells found in layer 2/3 of the rat medial prefrontal cortex (mPFC). Whole cell patch clamp recordings were used, in combination with selective antagonists, to isolate NMDAR-mediated currents carried by either NR2A or NR2B containing NMDA receptors. We found that the majority of NMDA current was carried by NR2A containing receptors in both interneurons and pyramidal cells from young (4-6 month old) animals, we carefully quantified the percentage of cells that exhibited detectable NR2B mediated currents, we calculated the ratio of NR2A:NR2B current in cells that displayed both components, and we evaluated the extent to which these values are altered by age. We found that ~40% of cells layer 2/3 interneurons tested in young animals had detectable NMDA current carried by NR2B containing NMDARs, which represented ~40% of total NMDA receptor mediated current in those cells. Interestingly, neither the percentage of NR2B positive interneurons, nor the NR2B:NR2A current ratio in those interneurons was altered in aged (20-24 month old) rats. In contrast, we found that the vast majority of young layer 2/3 pyramidal cells exhibited detectable current carried by NR2B containing NMDA receptors (representing ~34% of the total NMDAR mediated current), and that the percentage of NR2B positive pyramidal cells was significantly reduced by age. Interestingly, in aged cells that retained detectable NR2B current, the overall NR2A:NR2B ratio was not altered by age ($p=0.79$). Overall these observations indicate a prominent role for NR2A

containing NMDA receptors in cortical function, and are consistent with an age related loss of NR2B containing receptors in layer 2/3 of the rat mPFC that is largely restricted to a subset of cortical pyramidal cells.

Disclosures: **K.B. Kelly:** None. **J.L. Bizon:** None. **C.J. Frazier:** None.

Poster

552. Working Memory: Hippocampus and Cortex

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant F32AG051371

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McKnight Brain Research Foundation

Title: Stress reactivity predicts impaired working memory in aging: Vulnerability of GABAergic synapses

Authors: *J. A. MCQUAIL[1], M. M. BRUNER[1], C. M. HERNANDEZ, III[1], E. G. KRAUSE[2], B. SETLOW[3], D. A. SCHEUER[4], J. L. BIZON[1];
[1]Dept. of Neurosci., [2]Dept. of Pharmacodynamics, [3]Dept. of Psychiatry, [4]Dept. of Physiol. and Functional Genomics, Univ. of Florida, Gainesville, FL

Abstract: Normal aging is associated with impaired cognition, including working memory supported by the prefrontal cortex (PFC). Our prior work strongly implicates altered PFC glutamatergic and GABAergic signaling in age-related working memory impairment. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis also accompanies the aging process and it has been proposed that the cumulative effects of stress and concomitant glucocorticoid exposure over the lifespan exacerbate neural changes that mediate the emergence of cognitive deficits. As the PFC is enriched in glucocorticoid receptors, the present study tested the hypothesis that age-related differences in HPA function predict working memory ability and that psychogenic stress recapitulates adverse effects of aging on PFC glutamatergic and GABAergic signaling protein expression. First, we evaluated the relationship between working

memory and circulating corticosterone (CORT) in aged rats. Young adult (4-6 mo) and aged (22-24 mo) rats were characterized for working memory ability using a delayed response task. As in our previous work, working memory in aged rats was less accurate than young, although aged performance spanned a broad range with some aged rats performing similar to young (unimpaired) and others performing worse than young (impaired). Basal CORT measured across the diurnal cycle was greater in aged rats than in young but this elevation was not associated with working memory. When challenged with a stressor (1 h restraint), stress-induced CORT was positively correlated with working memory performance of aged rats. Next we determined the extent to which chronic variable stress alters expression of excitatory and inhibitory synaptic proteins in the PFC. Young adult rats were subjected to a 14-day randomized schedule of twice-daily stressors including insulin-induced hypoglycemia (Day 1 only), forced swim, novel environment, restraint stress and exposure to predator urine. While markers affiliated with excitatory signaling (NMDARs, VGluT1) were not reliably changed by stress, expression of GABA(B)R1a, a presynaptic GABA autoreceptor, and VGAT, the presynaptic vesicular GABA transporter, were significantly reduced in the PFC of stressed rats. Collectively, our findings reveal important interrelationships between aging, stress and PFC function and, further, identify a causal role for stress in PFC GABA signaling alterations that could contribute to impaired working memory. Ongoing studies will evaluate age- and stress-related changes in glucocorticoid receptors as well as the effects of synthetic glucocorticoid receptor agonists on working memory function.

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Poster

552. Working Memory: Hippocampus and Cortex

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Support: McKnight Brain Research Foundation

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UF Research Seed Opportunity Fund

Title: Velocity modulated hippocampal local-field potential across hippocampal lamina

Authors: *A. P. MAURER[1], C.-H. ELVIRA-MARTIN[1], S. N. BURKE[1], A. SHEREMET[2]; [1]Evelyn F. McKnight Brain Inst., [2]Univ. of Florida, Gainesville, FL

Abstract: The anatomy of the hippocampus is characterized by a laminar organization such that afferent input and intrinsic fiber projections are isolated in discrete layers. The layers of CA1, for example, are divided into stratum oriens, stratum pyramidale, stratum radiatum and stratum lacunosum-moleculare. The stratum lacunosum-moleculare region of CA1 neurons receives input from the layer III of the entorhinal cortex (Witter et al., 1988), nucleus reuniens (Wouterlood et al., 1990) and amygdala (Pikkarainen et al., 1999), while the stratum radiatum and oriens receive CA3 Schaeffer collateral and commissural fibers (Hjorth-Simonsen, 1973; Ishizuka et al., 1990). Moreover, different populations of interneurons target distinct hippocampal lamina with parvalbumin positive cells synapsing in the stratum pyramidale and oriens while the somatostatin positive cells target the stratum lacunosum-moleculare (Freund and Buzsaki, 1996) Theoretically, this anatomical organization should manifest as physiological differences in the local field potential. Using 32-site linear arrays, we investigated the rhythmic activity of the local-field potential as a function of depth in rats that were trained to perform a spatial alternation task. Specifically, as a function of the animal's running velocity we investigated theta (8 Hz), the theta harmonics (phase locked integer of the hippocampal theta rhythm; Sheremet et al., 2016), and the individual gamma rhythms (Colgin et al., 2009; Belluscio et al., 2012). Finally, we extended these analyses to determine the nature of cross-frequency coupling of oscillations both within and across hippocampal subregions.

Disclosures: A.P. Maurer: None. **C. Elvira-Martin:** None. **S.N. Burke:** None. **A. Sheremet:** None.

Poster

552. Working Memory: Hippocampus and Cortex

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UF Research Seed Opportunity Fund

Title: Broad neuronal population coding in hippocampus relative to piriform cortex during difficult olfactory discriminations

Authors: *S. A. JOHNSON[1,2], W. M. YODER[3], K. N. LUBKE[2], J. P. LISTER[6], A. P. MAURER[4], J. L. BIZON[2], S. N. BURKE[2,5];
[1]Dept. of Neurosci., [2]Dept. of Neurosci. / McKnight Brain Inst., [3]Dept. of Psychology, [4]Departments of Biomed. Engin. and Neurosci. / McKnight Brain Inst., [5]Inst. on Aging, Univ. of Florida, Gainesville, FL; [6]Dept. of Pathology & Lab. Med., Univ. of California Los Angeles, Los Angeles, CA

Abstract: The ability to discriminate between similar stimuli is fundamental to accurate encoding and retrieval of episodic memories. For visual and spatial domains, this ability has been linked to activity within hippocampal circuits. However, it is not yet known whether the hippocampus and its cortical inputs perform this role across all sensory modalities. The current study sought to determine the extent to which neuronal populations in perirhinal cortex (PRC) and CA3 are engaged by olfactory discrimination, relative to piriform and lateral entorhinal cortical regions associated with olfactory processing (Chapuis & Wilson 2013). Male F344 rats (N=6) were trained to perform a go/no-go olfactory discrimination task using aliphatic alcohols and aldehydes (C3-C8) as stimuli. We have previously shown performance on this task is inversely related to structural similarity of odorant molecules, such that difficulty of discriminations increases as difference in carbon chain length between odorants decreases (Yoder et al. 2014). Neuronal populations active during easy versus difficult discriminations were identified with cellular compartmental analysis of temporal activity by fluorescence *in situ* hybridization (catFISH), from which the activity history of neuronal ensembles can be inferred based on subcellular distribution of Arc mRNA (Guzowski et al. 2005). After initial training, rats performed similarly on easy (85.7% correct, SD=7.7%) and difficult trials (83.3%, SD=9%). Overall, more neurons were active during olfactory discrimination in the PRC (17.5%, SD=2%; $P < 0.006$) and CA3 region (21.8%, SD=5.8%; $P < 0.007$) relative to piriform cortex (11.3%, SD=2.7%). In the piriform cortex and PRC, the difference in proportion of neurons active during difficult relative to easy trials was not significantly greater than zero (piriform: $P = 0.08$, PRC: $P = 0.30$). Conversely, in CA3 there were more neurons active during difficult trials relative to easy trials (Difference in % Arc+ neurons=6.24, SD=2.7; $P < 0.05$). Our results demonstrate that the relative size of neuronal ensembles recruited across the piriform, PRC, and CA3 increases when rats are accurately discriminating between similar, but not distinct, odorants. These findings are consistent with prior implications of hippocampal activity in olfactory working memory (Kesner et al. 2011; Weeden et al. 2014), and support the hypothesis that the hippocampus is critical for the encoding of distinct representations of similar stimuli across multiple sensory modalities.

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Poster

552. Working Memory: Hippocampus and Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: R01DA036534

Thomas H. Maren Fellowship

Title: The medial prefrontal cortex is critical for the flexibility necessary for adaptive risky decision-making.

Authors: *C. A. ORSINI[1], S. C. HESHMATI[2], S. C. WALL[2], J. L. BIZON[2], B. SETLOW[2]; [1]UNIVERSITY OF FLORIDA, Gainesville, FL; [2]Univ. of Florida, Gainesville, FL

Abstract: Previous research shows that prefrontal cortex (PFC) damage mimics decision-making deficits observed in individuals suffering from substance use disorder (SUD). Thus, it is conceivable that impaired decision-making in SUD is due to alterations in PFC integrity. While the role of the medial PFC (mPFC; homologous to the primate dorsolateral PFC) has been elucidated in some forms of decision-making, its contributions to decision-making under risk of explicit punishment is unknown. To this end, we assessed the effects of mPFC manipulations in a rat model of risky decision-making [the "Risky Decision-Making Task" (RDT)], in which rats chose between a small, "safe" food reward and a large, "risky" food reward accompanied by ascending probabilities of mild footshock punishment. In Exp. 1, well-trained rats were tested in the RDT following mPFC inactivation with muscimol/baclofen (M/B) or vehicle. In Exp. 2, effects of intra-mPFC M/B or vehicle were assessed on a modified version of the RDT in which reward magnitudes associated with each lever were equated (such that rats chose between a small, safe reward and a small, risky reward). To determine whether effects of mPFC inactivation were due to behavioral inflexibility, a new cohort of rats was trained in a "descending" RDT in which the probabilities of punishment decreased across the session (Exps. 3-5). In Exp. 3, rats received intra-mPFC M/B or vehicle. In Exp. 4, rats received intra-mPFC amphetamine (AMPH; 0, 2, 10, 20 µg). Finally, in Exp. 5, rats received systemic injections of AMPH (0.0, 0.3, 1.0, 1.5 mg/kg). mPFC inactivation increased choice of the large, risky reward in the ascending RDT. When reward sizes were equated, choice behavior was not affected by mPFC inactivation, indicating no effects on sensitivity to punishment. In the descending RDT, mPFC inactivation decreased choice of the large, risky reward. Together, Exps. 1-3 suggest that the mPFC is necessary for adjusting choice behavior in response to changing punishment probabilities. In Exp. 4, similar to mPFC inactivation, intra-mPFC AMPH decreased choice of the large, risky reward in the descending RDT, suggesting mPFC monoamine neurotransmission is critical for flexible decision-making. Finally, and consistent with prior work in the ascending

RDT, systemic AMPH decreased choice of the large, risky reward in the descending RDT. Thus, it is also possible that enhancing mPFC monoamine neurotransmission potentiated the salience of punishment, causing a decrease in risk-taking. Future studies will dissociate these two interpretations and determine the roles of specific monoamine systems (dopamine vs. norepinephrine) to risky decision-making.

Disclosures: C.A. Orsini: None. **S.C. Heshmati:** None. **S.C. Wall:** None. **J.L. Bizon:** None. **B. Setlow:** None.

Poster

552. Working Memory: Hippocampus and Cortex

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Claude D. Pepper Older Americans Independence Center Scholar Award P30 AG028740

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Title: Stimulus modality affects recognition behavior during spontaneous object recognition and crossmodal object recognition tasks

Authors: *L. S. GAYNOR[1,2], J. MIZELL[1], K. T. CAMPOS[1], L. SANTACROCE[1], C. MCEWEN[1], D. K. CHETRAM[1], A. P. MAURER[1], R. M. BAUER[2], S. N. BURKE[1]; [1]Dept. of Neurosci., [2]Dept. of Clin. and Hlth. Psychology, Univ. of Florida, Gainesville, FL

Abstract: The time a rodent spends freely exploring a novel object compared to a previously encountered object has been used to measure recognition abilities (Ennaceur & Delacour, 1988). More recently, differences in behavior on these spontaneous object recognition (SOR) tasks have been used to quantify age-related deficits in object recognition and discrimination (Burke et al., 2010). In rodents, this cognitive process likely relies on sensory information obtained from both visual and tactile modalities and declines with age. Rodents, however, have an aversion to lighted areas, exhibiting reduced exploration under these conditions (Crawley & Goodwin, 1980;

Belzung et al., 1987). Moreover, the spatial resolution of the rat somatosensory system, which is less than 100 um (Morita et al., 2011; PMID: 21673811), is well suited to support object recognition. Therefore, SOR behavior, as detected by a rat's preference for novelty, may be more readily observed when animals use tactile information relative to visual. We tested young and aged rats' preferences for novel compared to familiar objects in a visual and a tactile SOR task with a 5 min delay between sample and test phases. Furthermore, to examine interactions between these modalities we used the Crossmodal Object Recognition task (CMOR) with and without multimodal pre-exposure to the sample object (Winters & Reid, 2010). This task tests the ability of rodents to identify objects based on visual features after familiarizing themselves with the object based on tactile cues only. In all 4 tasks, there was not a significant effect of age with a 5 min delay ($p > 0.1$ for all comparisons). Together, the young and aged rats showed a significant novelty preference in the tactile SOR task ($t[26] = 6.23$, $p < 0.001$), but not in the visual SOR task ($t[29] = 1.73$, $p = 0.1$). Additionally, both young and aged rats showed a significant novelty preference in the CMOR task with multimodal pre-exposure ($t[29] = 4.36$, $p < 0.001$), but not in the CMOR task without pre-exposure ($t[29] = 1.37$, $p = 0.2$). Together these data suggest that the superiority of the rat somatosensory system compared to the visual system results in preferential use of tactile over visual cues to guide crossmodal object recognition. In the CMOR with pre-exposure condition, rats are given the chance to encounter the test object's visual and tactile cues simultaneously, conceivably allowing the rat to incorporate both into a single, polymodal object representation in advance of the sample and test phase.

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Poster

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Claude D. Pepper Older Americans Independence Center Scholar Award (P30 AG028740

UF Research Seed Opportunity Fund

UF College of Medicine University Scholars Program.

Title: A rodent model of medial temporal lobe-dependent discrimination deficits in the elderly

Authors: *S. M. TURNER[1], L. A. SANTACROCE[1], S. A. JOHNSON[1], S. N. BURKE[1,3,4], A. P. MAURER[1,2];
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Abstract: Advanced age is associated with cognitive deficits that include impairments in object recognition and discrimination. In humans, the magnitude of these deficits is proportional to the difficulty of the discrimination. That is, elderly subjects show greater impairments when the stimuli to be discriminated have more features in common, relative to when they are more distinct (Yassa et al. 2011). The neurobiological underpinnings of these declines, however, have not yet been elucidated. This is due, in part, to the lack of rodent model for age-related discrimination deficits. The goal of these studies was to determine whether age selectively impairs the ability to distinguish between similar stimuli in a rat model of old age, and to test the role of the hippocampus. In order to systematically vary the difficulty of object discriminations, stimuli were created from LEGO© blocks. In experiment 1, young (6-10 m) and aged (26-30m) male Fischer 344 x Brown Norway hybrid rats were trained on an object discrimination task in which one pair of objects was visually distinct (easy), while the other pair shared features (difficult). Although young [$p<.05$] and aged [$p<.05$] rats took significantly longer to learn the difficult compared to the easy discrimination, the aged rats were selectively impaired relative to young on the difficult discrimination only [$p<0.01$]. In order to more closely replicate the experimental design of human studies, in experiment 2, rats were tested for their ability to correctly discriminate between a well-learned target object and 3 novel foil objects, each with increasing similarity to the target. Similar to the first experiment, aged animals were impaired relative to young on the difficult discriminations [$p<0.05$]. Finally, in experiment 3, a reversible inactivation in young rats was used to test the requirement of a functional hippocampus in performing the difficult discriminations. Inhibition of hippocampal neural activity resulted in a difficulty-dependent deficit similar to that observed in aged rats. These data suggest that advanced age impairs the ability to accurately discriminate between objects that share overlapping features, and this deficit may emerge from hippocampal dysfunction occurring in advanced age.

Disclosures: **S.M. Turner:** None. **L.A. Santacroce:** None. **S.A. Johnson:** None. **S.N. Burke:** None. **A.P. Maurer:** None.

Poster

552. Working Memory: Hippocampus and Cortex

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Claude D. Pepper Older Americans Independence Center Scholar Award

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the College of Medicine University Scholars Program

Title: The ketogenic diet as a therapeutic strategy for improving motor and cognitive functioning in a rodent model of senescence

Authors: *A. R. HERNANDEZ, K. CAMPOS, L. TRUCKENBROD, L. SANTACROCE, C. M. HERNANDEZ, Y. SAKARYA, J. A. MCQUAIL, A. P. MAURER, J. BIZON, C. CARTER, S. N. BURKE;
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Abstract: The loss of independence in the elderly can result from both physical and cognitive decline. These declines may be a consequence of body weight fluctuations, decreased glucose utilization, and increases in damage from reactive oxygen species. Currently, limited treatment options are available to prevent the myriad of age-related deficits many individuals experience. We propose the use of a ketogenic diet as a metabolic intervention for alleviating the symptoms of several aspects of aging. This high fat, low carbohydrate diet elicits a shift in the body's main energy source away from glucose towards the use of ketone bodies. Previous studies have shown that the ketogenic diet can reduce inflammation, increase oxidative capacity, and reverse aberrant neural activity. The ability of aged animals to enter and maintain ketosis, and the impact of this diet on body composition, however, has not been systematically examined. In this study, young and aged Fisher 344 x Brown Norway Hybrid rats were given 51 kcal/day of a nutrient matched ketogenic or control diet for a duration of 12 weeks. Young and aged rats in the ketogenic group maintained significantly higher levels of β-hydroxybutyrate and lower levels of glucose relative to the controls. While all rats were modestly calorically restricted (by ~15%), rats on a ketogenic diet had significantly less visceral fat than control rats ($p < 0.007$). Moreover, both young ($p < 0.003$) and aged ($p < 0.001$) ketogenic rats had significantly less brown adipose tissue compared

to age matched controls. Additionally, TD-NMR was used to determine body composition, revealing that there was a significant increase in the ratio of lean to fat mass in the aged rats on the ketogenic diet ($p < 0.003$) but not in aged controls ($p = 0.119$). Finally, aged rats on the ketogenic diet maintained grip strength over a 10-week period, while aged rats on the control diet showed a significant decline ($p < 0.05$). Future studies will determine the extent that the ketogenic diet can improve performance on a motor-cognitive "dual-task" that requires walking while using working memory, which is particularly sensitive to dysfunction in advanced age.

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Poster

552. Working Memory: Hippocampus and Cortex

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Title: Group II and Group III metabotropic glutamate receptors are required for normal working memory and are reduced in the aged rat prefrontal cortex

Authors: ***M. R. SCHWABE**[1], C. M. HERNANDEZ[1], J. A. MCQUAIL[1], B. SETLOW[2], J. L. BIZON[1];
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Abstract: Working memory refers to the short-term maintenance of information and this process is thought to require the persistent activity of excitatory pyramidal neurons within the prefrontal cortex (PFC). Metabotropic glutamate receptors (mGluRs) are localized to pre- (Group II and III) and post- (Group I) synaptic sites in the PFC, where they modulate synaptic transmission and neuronal excitability. As the role of PFC mGluRs in working memory is still not well-defined,

separate cohorts of rats were used to evaluate the effects of antagonists targeting receptors within each of the three major classes of mGluRs: Group I (MTEP, mGluR5 antagonist), Group II (LY341495, mGluR2/3 antagonist) and Group III (MMPIP, mGluR7 antagonist). Following surgical implantation of guide cannula targeting the medial PFC (mPFC, the rat homologue of primate dorsolateral PFC), rats were trained to baseline performance on an mPFC-dependent delayed response task. Drugs were then administered acutely into mPFC using a within-subjects, Latin square design, such that all subjects within a cohort received vehicle and three doses of drug, with a 48 hour washout period between successive doses. Using this design, both the mGluR 2/3 antagonist (LY341495) and the mGluR7 antagonist (MMPIP) impaired working memory relative to vehicle, whereas the mGluR5 antagonist (MTEP) had no effect. A second series of experiments then used Western blotting to evaluate expression of mGluR2/3 and mGluR7 in the mPFC of young (6 mo, n=6) and aged (24 mo., n=12) rats to explore the hypothesis that age-associated changes in PFC mGluRs contribute to the well-described working memory impairments that accompany the aging process. Expression of both mGluR2/3 and mGluR7 receptor proteins was significantly reduced in aged mPFC relative to young adults. Collectively, these findings show that presynaptic mGluRs are critical for normal working memory, and further, that loss of presynaptic mGluRs in the mPFC could contribute to attenuated working memory abilities in aging.

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Poster

552. Working Memory: Hippocampus and Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant R01DA036534(BS)

Title: Differential effects of D2 and D3 dopamine receptor ligands on risky decision-making

Authors: *S. L. BLAES, C. A. ORSINI, M. SPURRELL, J. L. BIZON, B. SETLOW;
Univ. of Florida, Gainesville, FL

Abstract: Regulation of decision-making is integral to daily life, and dysregulation in this process is evident in many psychiatric conditions including ADHD, schizophrenia and substance use disorders. In addition to impaired decision-making, these disorders share the common feature of dysregulated dopamine signaling. Indeed, dopamine signaling plays a critical role in

regulating multiple forms of decision making; however, the specific dopamine receptor subtypes involved are still not yet clear. Previous work from our lab identified a role for D2-like, but not D1-like, dopamine receptors in modulating decision- making involving risk of explicit punishment. The goal of the current experiments was to differentiate the roles of D2-like receptors (specifically D2 and D3) in risky decision-making, using a behavioral pharmacological approach.
Adult male Long-Evans rats were trained on a Risky Decision Making Task (RDT) in which they made discrete-trial choices between two levers, one which delivered a small, safe food reward and the other which delivered a large food reward accompanied by ascending probabilities (0-100%) of mild foot shock punishment. Once rats attained stable performance on the task, they received i.p. injections of multiple doses of amphetamine (indirect dopamine agonist), bromocriptine (D2/3 agonist), sumanirole (D2-selective agonist) and PD128907 (D3-selective agonist). Each drug was tested in a separate experiment, using a randomized, within-subjects design such that each rat received each dose of drug.
Consistent with previous work from our lab, both amphetamine and bromocriptine caused a significant dose-dependent decrease in choice of the large, risky reward. Surprisingly, sumanirole produced a trend toward an increase in choice of the large, risky reward. PD128907 caused a decrease in choice of the large, risky reward, but as this effect was evident irrespective of the probability of punishment, it is likely that it was due at least in part to impairment in reward magnitude discrimination. The results of these experiments thus far suggest that both D2 and D3 receptors are important for modulation of risky decision-making. Future experiments will focus on replicating these findings, as well as determining the effects of D2 and D3 receptor antagonists.

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Poster

552. Working Memory: Hippocampus and Cortex

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F32AG051371

UF HHMI Science for Life Award

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R01AG049711

Title: Age-related alterations in working memory and intertemporal choice in Fischer 344 x Brown Norway hybrid rats.

Authors: *L. M. VETERE[1], C. A. ORSINI[2], J. A. MCQUAIL[3], S. N. BURKE[3], B. SETLOW[2], J. L. BIZON[3];
[2]Psychiatry, [3]Neurosci., [1]Univ. of Florida, Gainesville, FL

Abstract: The ability to evaluate the benefits and potential consequences associated with a choice and to make effective decisions is critical for maintained independence across the lifespan. Poor decision making is associated with impaired prefrontal cortical-dependent cognition across a variety of clinical conditions. However, despite the fact that prefrontal cortical function declines in aging, aged individuals generally show an enhanced ability to delay gratification as is evident by less discounting of delayed rewards in intertemporal choice tasks. The current study was designed to evaluate relationships between two aspects of prefrontal cortical-dependent cognition (working memory and cognitive flexibility) and intertemporal choice in young (6 mo.) and aged (24-28 mo.) Fischer 344 X Brown Norway hybrid rats. First, young and aged rats were tested on a food-motivated intertemporal choice task in which they chose between a small reward available immediately and a large reward available following variable delays ranging from 0-60 seconds. Overall, aged rats showed attenuated discounting of delayed rewards in this task compared to young (i.e., enhanced preference for large, delayed over small, immediate rewards). These same rats were then tested on a delayed response working memory task in which they had to remember the location of a response lever over delays ranging from 0-24 seconds. Aged rats were significantly impaired on the delayed response task compared to young, although among aged rats, those with better working memory tended to show the greatest ability to delay gratification on the intertemporal choice task. Finally, the rats were tested on a set-shifting task in which they had to shift between two response rules in order to receive food rewards. Performance on the set-shifting task did not predict intertemporal choice among aged rats. These data indicate that impairments in working memory and cognitive flexibility cannot account for the robustly attenuated discounting of delayed rewards observed in aged subjects.

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Poster

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NSF Graduate Research Fellowship Program DGE-0802270

Diversity Supplement to NIH grant R01AG029421

Title: Biochemical evidence for altered glutamatergic signaling in the aged prefrontal cortex: contribution to impaired behavioral flexibility

Authors: *C. M. HERNANDEZ, III[1], B. S. BEAS[1], J. A. MCQUAIL[1], B. SETLOW[2], J. L. BIZON[1];
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Abstract: Behavioral flexibility, or the ability to adjust response strategies when faced with changes in the environment, is critical for normal adaptive behavior. Behavioral flexibility is mediated by the prefrontal cortex (PFC) and is compromised in schizophrenia and other clinical conditions characterized by cortical hyperexcitability. This aspect of PFC-mediated cognition can also become impaired in aging and previous work from our laboratory has identified attenuated GABAergic signaling as a contributor to impaired behavioral flexibility among aged rats. The goal of the current study was to extend these investigations to determine the relationship between age-associated alterations in medial PFC (mPFC) expression of proteins involved in glutamate signaling and impaired behavioral flexibility. Young (6 mo, n = 6) and aged (24 mo, n = 12) Fischer 344 rats were first trained on a mPFC-dependent set-shifting task that assesses behavioral flexibility. Two weeks after the conclusion of testing, the mPFC was micro-dissected, fractionated, and the expression of synaptic proteins involved in glutamatergic signaling was evaluated using Western blotting. Several presynaptic proteins important for the regulation of synaptic glutamate were significantly reduced in expression in the aged mPFC. These included Group II (Grm 2/3) and Group III (Grm4, Grm7) metabotropic glutamate receptors, as well as the excitatory amino acid transporter EAAT1. While expression of these presynaptic proteins did not reliably predict behavioral flexibility among aged rats, they were

suggestive of increased glutamate availability at mPFC synapses. Several post-synaptic proteins (NR1, PSD-95, and Grik2) were selectively reduced in aged rats exhibiting impaired behavioral flexibility relative to the young cohort. It is notable that attenuated expression of both NMDA and kainate receptors has been strongly linked to impaired cognition across a variety of conditions. Moreover, blockade of NMDARs in the PFC generally produces increased excitation, supporting the functional role of these receptors in regulating interneuron activity. As such, the overall pattern of changes in protein expression provides evidence for cortical hyperexcitability in aged rats with impaired behavioral flexibility.

Disclosures: C.M. Hernandez: None. **B.S. Beas:** None. **J.A. McQuail:** None. **B. Setlow:** None. **J.L. Bizon:** None.

Poster

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Title: Neural population dynamics of contextual coding in LEC

Authors: *A. TSAO, L. LU, J. SUGAR, E. I. MOSER, M.-B. MOSER;
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Abstract: Recent work has demonstrated that lateral entorhinal cortex (LEC) is involved in encoding context. However, the details of how this occurs and the underlying mechanisms are not well understood. Here we take a population-level approach to understanding the details of how context is encoded in LEC, using tetrode recordings in rats across multiple experimental paradigms. In our first set of experiments, we change the wall color of the recording environment, which has been shown to induce rate-remapping in the hippocampus which is dependent on LEC function. In particular, we alternate between white and black walls 6 times in order to more fully capture contextual coding dynamics in LEC. In our second set of experiments, we introduce an object into the recording environment in the middle of the

recording session, in order to test the generality of contextual coding in LEC. Population-level analysis of LEC activity in both of these experiments reveals robust contextual coding, even in the relative absence of such information at the single-cell level, as context can be accurately predicted using a classifier trained on the entire recording population. In addition to context, trial order can also be accurately predicted, suggesting that LEC encodes both environmental and temporal context simultaneously. Population vector analysis verifies that temporal coding is not merely due to drift in the neural population. We are currently exploring the population dynamics present in these experiments, including those occurring at the contextual switches. In our final set of experiments, we train rats to perform a continuous-alternation task in order to test contextual coding in a behaviorally meaningful situation. We find that the neural trajectories for left- and right-turn trials are well-separated throughout the maze except for the last half of the central arm, and that the distance between left-and right-turn neural trajectories increases significantly at the turning points of the maze, suggesting that LEC codes for behavioral context by keeping track of the animal's actions.

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Poster

552. Working Memory: Hippocampus and Cortex

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Topic: H.01. Animal Cognition and Behavior

Title: The impact of NMDA and NR2B antagonists on working memory and c-fos expression in rats.

Authors: *L. M. FLEMING[1], S. KRIEGER[2], R. FRAWLEY[2], T. SONGTACHALERT[2], L. LUNDEGARD[2], P. IVAIN[2], C. CHADWICK[2], K. FISCHER[2], F. MENNITI[3], R. GRAHN[2]; [1]Yale Univ. Neurosci. Program, New Haven, CT; [2]Connecticut Col., New London, CT; [3]MindImmune Therapeutics, Inc, Kingston, RI

Abstract: The NMDA receptor plays a role in mental processes ranging from mood regulation to memory formation. Ketamine, a non-selective NMDA antagonist, has both rapid antidepressant effects and side effects similar to the symptomology of schizophrenia. Another link between these disorders is working memory, which relies on the NMDA receptor and specifically NR2B-subunit function. We investigated the effects of acute and long-term administration of ketamine and an NR2B-specific antagonist (traxoprodil) on working memory in rats. Rats were tested with a spatial working memory procedure on the eight-arm radial arm maze after acute and repeated

doses of each drug. After the animals' final day of testing on the maze, brains were extracted for immunohistochemistry staining using a c-fos antibody. The results showed a trend indicating that animals repeatedly administered ketamine performed better on the working memory task than those administered saline or traxoprodil. The animals administered ketamine also showed significantly lower c-fos expression in areas of their frontal cortex compared to animals receiving saline and traxoprodil. These results indicate that the long-term effects of ketamine do not appear to be the results of its action on the NR2B subunit. These findings have positive implications for the therapeutic use of low doses of this drug class for depression and hints at dose-related differences in the effects of repeated ketamine administration.

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Poster

552. Working Memory: Hippocampus and Cortex

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Topic: H.01. Animal Cognition and Behavior

Support: UF internal funds

Title: Evaluating the role of mGluR5 in post-cocaine working memory deficits and relapse

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Abstract: Chronic cocaine abuse produces motivational and cognitive deficits that might be rooted in abnormal function of circuitry that includes the prefrontal cortex (PFC) and the nucleus accumbens (NAc). While diminished activity of the PFC during post-cocaine abstinence has been linked to deficits in working memory and cognitive flexibility, over activation of PFC-to-NAc glutamatergic pathway permits relapse to drug-seeking. Systemic administration of metabotropic glutamate receptor 5 (mGluR5) antagonists attenuate relapse, but prolonged blockade of mGluR5 might exacerbate post-cocaine PFC dysfunction. Conversely, mGluR5 agonists, while potentially beneficial for treating PFC dysfunctions, may increase the risk of relapse. Here we wanted to evaluate the effects of prolonged mGluR5 inhibition or activation on

working memory performance and reversal learning following cocaine or saline self-administration. We used a novel combination of cocaine self-administration followed by an operant delayed match/non-match to sample task (DMS/DNMS). In the DMS task, each trial consists of a sample phase, delay period and choice phase. In the sample phase, either the left or right lever results in a sucrose pellet. In the choice phase, choosing the same lever presented in the sample phase yields sucrose delivery. Performance with variable delays assesses working memory. In the DNMS task, the rule is switched in which choosing the opposite side lever as the one presented in the sample phase results in a reward. Upon reaching stable performance over a block of five days, rats received daily systemic injections of the mGluR5 antagonist MTEP or the mGluR5 positive allosteric modulator CDPPB over a block of five days followed by a washout period of five days. We found that MTEP significantly impaired, while CDPPB mildly enhanced, cognitive performance in saline self-administering rats. We predict that prolonged use of MTEP will be damaging to cognitive function, but CDPPB may treat cognitive deficits in rats with a history of cocaine without negatively affecting relapse.

Disclosures: **C.M. Gobin:** None. **M. Schwendt:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: Grinnell College

Title: Effect of high-fat diet on consolidation of object location memory and hippocampal leptin receptor levels

Authors: T. OMURA, J. ZIONTZ, *A. L. TRACY;
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Abstract: The increasing global prevalence of obesity in the 21st century has been associated with a variety of life-threatening diseases. In rodent models, obesity induced by a high-fat diet (HFD) has also been shown to impair learning and memory compared to lean animals. The hormone leptin, produced in adipose tissue, is primarily known as a regulator of food intake behavior in the hypothalamus but is also implicated in altering learning/memory behavior by affecting the hippocampus. In the present study, rats were fed either a HFD (40% fat, 4.54 kcal/g) or a standard chow diet (4.4% fat, 3.93 kcal/g) for 10 weeks. Spatial memory was assessed using an object location recognition (OLR) behavioral assay. Rats were first allowed to

explore the empty testing arena (habituation), and, in a separate training session, placed in the arena with two identical objects to investigate, positioned at locations A and B (acquisition). Rats were then removed and one of the objects was moved to novel location C while the other remained in its original location A. After a period of 5 minutes, animals were placed back into the arena and allowed to freely explore the objects in this configuration (first retention). After 1 hour, animals were placed a final time into the arena with objects now placed in original location A and novel location D (second retention). Interaction time with each object was measured and compared across diet condition and retention delay interval. After the first retention interval (t = 5 min), there were no significant differences in demonstrated learning between diet conditions. After the second retention interval (t = 1hr), only animals on a standard chow diet demonstrated learning by exploring the object in the novel location more than the object in the familiar location, while animals on the HFD not differentially explore the two objects. This impaired performance at a longer retention interval suggests that HFD-induced obesity impairs consolidation of spatial memory in a rodent model. Hippocampal leptin receptor was quantified via Western blot and correlated with OLR performance.

Disclosures: **T. Omura:** None. **J. Ziontz:** None. **A.L. Tracy:** None.

Poster

552. Working Memory: Hippocampus and Cortex

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 552.17/LLL19

Topic: H.01. Animal Cognition and Behavior

Title: Toxicological acute effect of genus of kalanchoe blossfeldiana plant over density on neuronal prefrontal cerebral cortex and short-term memory in rats male wistar

Authors: *P.-V. MARIA ISABEL[1], P. GUDIÑO-GUEVARA[2], L. HUACUJA-RUÍZ[2], M. MIRANDA BELTRAN[2];
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Abstract: Historically, the traditional medicine has been used significantly to preserve health, prevent and treat various diseases. The safety should be a decisive criterion in the selection of herbal medicines for use in health services. Whole plants, as well as each of its parts, natural extracts and pure phytochemicals should be subject to a selection process, chemical analysis, clinical trials and regulatory measures to evaluate its potential effect on the body. For pure phytochemicals, procedures should be an identical those who apply to synthetic drugs. The aim of this study was to evaluate the effect of the *Kalanchoe blossfeldiana (K.b)* on the neuronal density of the layer III of the cerebral prefrontal cortex (CCFP) correlative with the execution of

a test short-term memory. Three groups of male wistar rats were used; 1) control, 2) vehicle and 3) rats received 5g/kg of weight-*K.b*., Lyophilised natural juice of *K. blossfeldiana* was used for the behavioral evaluation in Biel's Labyrinth of firm ground. The CCFP was processed with the hematoxylin-eosin technique. The results show alterations in the execution of the task after receiving treatment, however histologically no changes which could result from possible physiological alterations at the molecular level change behavioral activity without getting to cause observable effects on cell histology.

1. **Valdés, J. L. G.,** y Torrealba, F., (2006). **The medial prefrontal cortex controls the behavioral and vegetative arousal. Implications for behavioral disorders**, Reviews Chilena Neuro-Psiquiat. 44(3):195-204.

2. **Vettorazzi, G.,** (2006). Tests required for toxicological assessment of chemicals. International Program on Chemical Safety. World Health Organization. descriptive note. 67-77.

3. **Vorhees, Ch. V**., (1986). Methods for assessing the adverse affects of foods and other chemicals on animal behavior. Nutritions Reviews Supplement. 44:185-92.

Disclosures: P. Maria Isabel: None. **P. Gudiño-Guevara:** None. **L. Huacuja-Ruíz:** None. **M. Miranda Beltran:** None.

Poster

552. Working Memory: Hippocampus and Cortex

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 552.18/LLL20

Topic: H.01. Animal Cognition and Behavior

Support: NIH R01 DA026297

NIH R01 EY017658

Title: Corticostriatal contributions to working memory: single-unit and local field potential dynamics during working memory maintenance and updating

Authors: *D. HUIE[1], A. PAULK[2], T. M. HERRINGTON[3], E. ESKANDAR[2];
[2]Neurosurg., [3]Neurol., [1]Massachusetts Gen. Hosp., Boston, MA

Abstract: Background: Working memory is the capacity to briefly hold and manipulate information, which is robustly maintained against distraction, but may also be flexibly updated

when necessary. While working memory storage has primarily been ascribed to prefrontal cortex, the basal ganglia is thought to play a gatekeeping role, permitting certain stimuli to access and change working memory while protecting working memory from distracting input. Here, we recorded from neurons in the dorsolateral prefrontal cortex and caudate nucleus of two rhesus macaques while they engaged in two visual non-spatial working memory tasks.
Methods: Microelectrode recordings were performed in rhesus macaques within the anterior caudate nucleus and dorsolateral prefrontal cortex while subjects engaged in either a distractor chain task or one-back task. During task performance, the animal was first shown a cue indicating the task to be performed, followed by a variable, unknown-length series of non-repeating images drawn from a pool of 16 familiar images. Following a short delay, the subject was then shown two image choices. The correct answer was either the first image in the chain (distractor chain) or the last image (one-back).
Results: Preliminary field potential analysis demonstrates separable dynamics in the dorsolateral prefrontal cortex and caudate nucleus during the two tasks. We also found variation in spiking activity at the single-unit level that was dependent upon both image identity and position within the image chain, as well as the current task rule. Ongoing analyses will further investigate single-unit, field potential, as well as spike-field dynamics within the corticostriatal network during task performance.
Discussion: Our current results indicate that the varying demands of the one-back and distractor chain tasks result in measurable differences in neural activity within the dorsolateral prefrontal cortex and caudate nucleus. Ongoing analysis will further test the role of the corticostriatal circuit in working memory function.

Disclosures: **D. Huie:** None. **A. Paulk:** None. **T.M. Herrington:** None. **E. Eskandar:** None.

Poster

552. Working Memory: Hippocampus and Cortex

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 552.19/LLL21

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI 26350988

KAKENHI 26115518

Title: Real-time change of neural activity in hippocampal CA1 after the experienced episodes; restraint stress and first encounters with female, male, and object

Authors: *J. ISHIKAWA[1], D. MITSUSHIMA[2];
[1]Yamaguchi Univ. Grad Sch. Med., Ube, Japan; [2]Yamaguchi Univ. Grad. Sch. of Medicine., Ube, Japan

Abstract: The hippocampal CA1 is necessary to maintain experienced episode in many species including humans. To monitor the process of the episode, we used freely moving male rats to record neural activity of CA1 neurons before, during, and after a specific episode. The rats were experienced either the restraint stress, or the first encounter with a female, a male, or an object for 10 min. Although spontaneous firing rate was relatively low in their habituated home cage, we observed spontaneous high frequency firing (super burst: > 600 ms duration with 43 ~ 129 Hz frequency) during and soon after the episodes. The episodes with female or restraint consistently induced the super bursts, while the episode with male or object induced the events inconsistently. Then, minutes after the episode, CA1 neurons exhibited short term (≈ 50 ms) high frequency ripple-like synchronized firing. Each ripple-like event exhibited different shape, duration, and frequency, showing a wide diversity. Moreover, simultaneous monitoring of the firing with theta wave further revealed the phase-locked relation of ripple-like events with hippocampal theta wave ($\varphi \approx 180°$). Total duration of super bursts, the number of ripple-like firing, and the timing of increase in the ripple-like firing were different among the 4 different episodes. Moreover, the rats exhibited more and longer super bursts showed more ripple-like firing after the episodes. To further examine the synaptic plasticity after the episodes, we made acute brain slices 30 min after the episodes. Compared with inexperienced controls, the episodes with female, male, and restraint significantly increased the amplitude of miniature EPSCs and IPSCs, while the episode with novel object increased the amplitude of miniature IPSCs only. Karnel density estimation of further demonstrated that the different episode promotes different diversity at excitatory and inhibitory synapses. Experienced episode promotes a wide diversity of excitatory/inhibitory CA1 synapses that may depict neuron specific outputs to process the experienced episodes. Spontaneous super bursts and following ripple-like firing may be necessary for the processing of experienced episodes in male rats.

Disclosures: J. Ishikawa: None. **D. Mitsushima:** None.

Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

Location: Halls B-H

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Program#/Poster#: 553.01/LLL22

Topic: H.01. Animal Cognition and Behavior

Support: Kaken-hi (15H05569)

Keken-hi (15H01417)

Title: Goal-directed firing of hippocampal cells in a two-dimensional open field

Authors: *Y. AOKI[1], H. IGATA[2], T. SASAKI[2], Y. IKEGAYA[2];
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Abstract: The hippocampus is composed of place cells that fire preferentially when an animal visits a restricted area of an environment. A widely used behavioral task for characterizing place cells is a foraging task in which rats freely explore an open two-dimensional space in search of randomly dispersed food. In this task, firing of place cells tends to show little dependence on moving direction. On the other hand, in spatial tasks on a radial arm maze and a linear track where animals develop goal-directed behavioral strategy, place-selective firing of hippocampal cells is strongly biased to a specific moving direction. Taken together, the hippocampal circuit employs distinct encoding modes for space depending on ongoing task demands and the presence of goals. In this study, we recorded firing patterns of hippocampal cells while rats performed a goal-related task and a random foraging task in an identical open field. In the goal-related task, rats were required to run toward a light-cued goal to obtain a food reward in the field. Enhancing the degree of freedom of moving directions by utilizing the two-dimensional space allowed us to analyze animal's trajectories from various provenance toward goals, including errors, which could not be parsed in a stereotyped one-dimensional path, such as a linear track. We found a certain population of hippocampal cells in the goal-directed task fired only at the time when rats approached to a future goal or left from a previous goal independent of moving directions. Some of the goal-directed place-selective firing patterns differed from those observed at the same locations in the foraging task. We will further analyze what proportion of the cells share distinct spatial coding patterns, to what extent their firing fields are affected by goal locations, and whether goal-directed firing patterns are associated with task performance. The evidence will advance our understanding of how place-selective firing of hippocampal cells is modified by goal-directed movement in an open field.

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Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

Location: Halls B-H

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KAKENHI (15H04265) to T.F.

KAKENHI (26115504, 25111703 and 16 21115504) to J.N.

Regional Innovation Cluster Program (City Area Type, Central Saitama Area) to J.N. and M.O.

Title: Temporal coding of reward event by subpopulations of hippocampal CA1 pyramidal neurons

Authors: K. MIZUTA[1], M. SATO[1,2,3], Y. SEKINE[1], M. KAWANO[1], T. ISLAM[1], R. TAKAMURA[1,4], T. MASUMOTO[1], T. TAKEKAWA[1,5], M. OHKURA[3], T. FUKAI[1], J. NAKAI[3], *Y. HAYASHI[1,3];
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Abstract: Hippocampus plays an essential role in memory formation for space and events. However, it is not well understood how hippocampal microcircuits are reorganized during the formation and retention of memory for a particular place associated with value such as a reward event. Although it has been reported that an event-related memory can be recalled by activation of a sparse but specific ensemble of hippocampal neurons (Tonegawa *et al*., Nature, 2012), it remains a possibility that a temporal firing sequence within the event-specific ensemble can recall the event-related-memory robustly. Furthermore, the stability of these ensembles over days and changing event conditions should be investigated. To address these questions, we conducted calcium imaging of hippocampal CA1 neurons in mice that express the fluorescent calcium sensor protein G-CaMP7. During recording, mice were head-fixed under a two-photon microscope while performing a reward-related memory task in virtual reality. Three colored zones were placed along a virtual linear track and reward was given in one of them. Mice were required to remember the correct reward zone and learn to stay there for 2 seconds to receive reward, as a delay condition. With training, mice learned to stop and stay in the reward zone to get reward, eventually reaching a success rate up to 70%. In a single session, about 10-15% of recorded neurons demonstrated time-locked activity while the mice stayed at the reward zone. Among recording sessions over days, the temporal neural activity within the reward zone remained stable for a subpopulation of neurons. When the reward zone was changed among the three before mentioned zones, 2% of all identified cells kept responding in the previous reward

zone, while other subpopulations encoded the present reward zone with specific temporal firing pattern. Moreover, when the delay condition to the reward was omitted or when reward was not given, the temporal activity of neurons representing the reward event was suppressed. However, the activity pattern of some neurons recovered after the reward condition was reinstated. These results raise an intriguing possibility that an event such as reward is coded with sequential firing within groups of CA1 neurons, and suggest a probable mechanism for robust recall of event-related-memory.

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Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: JST PRESTO and KAKENHI (21800091, 24700403, 25116528 and 26115530) to M.S.

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Regional Innovation Cluster Program (City Area Type, Central Saitama Area) to J.N. and M.O.

Title: Preferential stabilization of behaviorally relevant spatial representations in the hippocampal place map

Authors: ***M. SATO**[1,2], K. MIZUTA[1], T. ISLAM[1], M. KAWANO[1], T. TAKEKAWA[3], D. GOMEZ-DOMINGUEZ[1,4], H. YAMAKAWA[1,5], M. OHKURA[6,7], T. FUKAI[1], J. NAKAI[6,7], Y. HAYASHI[1,6,8,9];

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Abstract: The hippocampus plays a crucial role for formation and consolidation of spatial memory, and location-specific activity of hippocampal neuronal populations provides a mnemonic representation of the animal's environment. Although previous studies have demonstrated that cells that encode locations of behavioral relevance, such as reward, are disproportionately overrepresented in hippocampal place maps, how this overrepresentation is established is poorly understood. Thus, we chronically imaged formation and stabilization of hippocampal CA1 place cell maps using two-photon calcium imaging in Thy1-G-CaMP7 transgenic mice during training on a linear track task in a head-fixed virtual environment. A salient visual landmark and reward delivery were associated as two types of behavioral relevance with two distinct locations in the virtual linear track. Fraction of time spent running and fraction of virtual place cells increased as training progressed. Overrepresentation of these locations was evident from the early phase of training, although place cell maps were highly variable during this period. These unstable maps were increasingly stabilized by repeated training to an extent correlated with behavioral performance. Cells that encoded these behaviorally relevant locations showed higher inter-session place field stability than place cells that encoded other locations, whereas net formation of place cells from non-place cells and recruitment of place cells that encoded other locations were not significantly biased toward these locations. These findings indicate that representations of behaviorally relevant locations form more persistent traces in hippocampal cognitive maps and that such preferential stabilization serves as an underlying mechanism for the hippocampal overrepresentation of these locations.

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Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NSF grant IOS 1051919

Title: Light modulates spatial learning and memory in a diurnal rodent, the nile grass rat (arvicanthis niloticus).

Authors: *J. E. SOLER, A. NÚÑEZ, L. YAN;
Michigan State Univ., East Lansing, MI

Abstract: The effects of light on cognitive function have been well-documented in human studies, with brighter illumination improving cognitive performance in schoolchildren, healthy adults and patients in early stages of dementia. However, the neural mechanisms through which ambient light modulates cognitive functions are not well understood. There are substantial differences in how light affects nocturnal and diurnal species e.g., light induces sleep in nocturnal mammals and wakefulness in diurnal ones. An animal model with a diurnal pattern of general activity, like that of our species, is critical for uncovering how light modulates cognition in humans. The present study utilized the diurnal grass rat (Arvicanthis niloticus) to investigate the effects of ambient light on hippocampal function. Grass rats were housed in either a 12:12hr bright light-dark (briLD, 1000 lux) or dim light-dark (dimLD, 50 lux) cycle. After 4 wks, the dimLD group showed impaired spatial memory in the Morris Water Maze (MWM) task, as revealed by increased latencies to locate the platform during the training period and less time spent searching for the platform in the goal quadrant during the testing period. When grass rats in the dimLD were rehoused under briLD condition for another 4 wks, their performance in MWM significantly improved. The results collectively suggest that light modulates hippocampal-dependent spatial memory in the diurnal grass rats. In addition to the behavioral analysis, hippocampal plasticity was also assessed by measuring brain-derived neurotrophic factor (BDNF) and dendritic spine density using immunohistology and Golgi staining, respectively. Grass rats in the dimLD condition exhibited reduced hippocampal BDNF expression, especially in the CA1 sub-region, and approximately a 50% reduction in dendritic spine density in CA1 apical dendrites. When dimLD animals were transferred to the briLD condition and kept there for 4 weeks, the hippocampal BDNF and dendritic spine density significantly increased. The results illustrate that not only does light intensity affect cognitive function, but that it also impacts hippocampal plasticity. These studies serve as a starting point to further understand the neural pathways mediating the effects of light on cognition.

Disclosures: **J.E. Soler:** None. **A. Núñez:** None. **L. Yan:** None.

Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 553.05/LLL26

Topic: H.01. Animal Cognition and Behavior

Title: Optimal population coding by mixed-dimensionality neurons in the head-direction system of bats

Authors: *A. FINKELSTEIN[1], N. ULANOVSKY[1], M. TSODYKS[1], J. ALJADEFF[2]; [1]Weizmann Inst. of Sci., Rehovot, Israel; [2]Univ. of Chicago, Chicago, IL

Abstract: Ethologically-relevant stimuli are often multidimensional. Recently we found that head-direction is encoded in the bat brain by two major neuronal classes: 'pure' 1D cells, which encode azimuth or pitch independently; and 'conjunctive' 2D azimuth-by-pitch cells, which encode head azimuth and pitch jointly. Together, these two populations form an apparently redundant representation. Here, we show that such mixed-dimensionality coding by pure and conjunctive populations is in fact advantageous for efficient representation of head-direction in different behaviorally-relevant regimes. Specifically, we found that encoding by conjunctive cells is more robust when the stimulus changes quickly, whereas pure cells represent the stimulus more efficiently on slower timescales. More generally, our results suggest that the optimal dimensionality of neuronal tuning-curves can strongly depend on population size and on the system's dynamic variables, such as the time available for decoding - which might explain why mixed-dimensionality representations are common in sensory, motor, and higher cognitive systems across species.

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Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

Location: Halls B-H

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Program#/Poster#: 553.06/LLL27

Topic: H.01. Animal Cognition and Behavior

Title: Towards bat hippocampal recordings in large-scale environments

Authors: *T. ELIAV, L. LAS, N. ULANOVSKY;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Over the last forty years, hippocampal electrophysiological research has typically focused on spatial representations in small laboratory environments. Nothing is known about hippocampal neural codes on large spatial scales - in environments spanning hundreds of meters or kilometers - the scales of natural navigation by rodents and other mammals. Here we aim for the first time to develop a unique recording setup, including a large-scale ethologically relevant environment, which will allow us to address this fundamental question. We are using the Egyptian fruit bat as our animal model, because (i) bats are excellent navigators over large natural spatial scales, and (ii) bats were shown to have rodent-like hippocampal spatial representations in small laboratory environments, during crawling and flight behaviors. So far, we took the following steps: First, we developed an on-board wireless neural-logging system, which allows recording single units over unlimited distances. Second, we built a 200-m long tunnel where bats can fly freely. Third, to track the bat's position we are utilizing an RF localization device that measures distances to an antenna-array - yielding spatial accuracy of ~10-cm, much better than GPS. Preliminary experiments showed that bats fly volitionally back-and-forth along the tunnel - up to 100 laps per session (20-km total flight distance) - exhibiting very high flight speeds of ~8 meters/sec. We hypothesize two possibilities for hippocampal coding of large-scale environments: (i) enlargement of place-fields to dozens of meters, versus (ii) hundreds of small place-fields for each place-cell. Here we will present the first neural recordings that directly test these hypotheses.

Disclosures: **T. Eliav:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 553.07/LLL28

Topic: H.01. Animal Cognition and Behavior

Title: 3D grid cells and border cells in flying bats

Authors: *G. GINOSAR, A. FINKESTEIN, A. RUBIN, L. LAS, N. ULANOVSKY;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Grid cells exhibit spatially-periodic firing fields, and are thought to be important for navigation. When recorded in animals moving on a 2D plane, the cells fire when the animal passes through the vertices of a hexagonal grid spanning the 2D environment. Despite extensive research on 2D grid cells, there is an ongoing debate regarding the function of these fascinating neurons - namely, whether they encode the position of the animal or the distance it travelled. Moreover, many animals navigate through 3D space, but no studies to date have attempted to characterize the 3D volumetric firing of grid cells. Here, we conducted experiments in flying bats to elucidate the grid code in 3D - and found that our results provide also surprising insights into the debate on grid function in 2D. We trained Egyptian fruit bats (Rousettus aegyptiacus) to fly in a flight-room, while we wirelessly recorded single-neuron activity in medial entorhinal cortex. We found several classes of 3D spatially tuned neurons, including 3D border cells and 3D head direction cells. Importantly, these recordings revealed grid-like structures in the 3D firing-rate maps of some cells, which exhibited multiple firing-fields. The spacing between firing-fields was more variable than in perfect synthetic 3D lattices, but was substantially less variable than for randomly-distributed fields - namely, 3D grid-cells seem to exhibit a rather fixed distance scale, without forming a global lattice. These preliminary data shed new light on the function of grid cells, because the global lattice arrangement is a key requirement for representing position using the widely-proposed grid-based modulo code. Conversely, the fixed inter-field spacing observed in our data might be suitable for the encoding of distance travelled.

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Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 553.08/LLL29

Topic: H.01. Animal Cognition and Behavior

Title: Vectorial representation of spatial goals in the hippocampus of bats

Authors: *A. SAREL, A. FINKELSTEIN, L. LAS, N. ULANOVSKY;
Dept. of Neurobio., Weizmann Inst. of Sceince, Rehovot, Israel

Abstract: Navigation, the ability to reach a desired destination, requires the knowledge of the position and direction to the goal. Decades of research have focused on the representation of the current location and orientation of the animal, revealing place cells, grid cells and head-direction cells. However, a fundamental question that remains unanswered is how spatial goals are represented in the brain - which is crucial for understanding the neural basis of goal-directed

navigation. To address this question, we trained three Egyptian fruit bats to fly towards landing-sites (defined as 'goals'), while the activity of single neurons from hippocampal area CA1 was recorded. We found a subpopulation of hippocampal neurons that exhibited rather narrow angular tuning to the direction of the goal. This goal-direction tuning was highly stable, over both short and long timescales, and was independent of the place tuning. Further, in two of the three bats we conducted another session in which we positioned the goal behind a curtain, and found many neurons tuned to this hidden goal - suggesting that the goal-direction representation is memory-based rather than sensory-based. In addition, we also found hippocampal neurons that encoded the distance to the goal, often in conjunction with goal-direction tuning. Taken together, our results suggest the existence of goal-direction and goal-distance signals in the bat hippocampus - a vectorial representation that could support goal-directed navigation.

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Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

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Program#/Poster#: 553.09/LLL30

Topic: H.01. Animal Cognition and Behavior

Title: Representation of conspecifics by bat hippocampal place cells

Authors: *D. B. OMER, N. ULANOVSKY, L. LAS;
Dept. of Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: In animals living in social groups, learning can be facilitated by observing the behavior of conspecifics (observational learning). In particular, it is important for social animals to know the spatial location of conspecifics, both because they need to know the locations of socially-dominant animals, and for purposes of group navigation. How is the location of other animals represented in the brain? Here, we addressed this question by studying bats - highly-social flying mammals that excel in observational-learning and are also outstanding navigators. We designed an observational-learning task for Egyptian fruit bats (*Rousettus aegyptiacus*), where animals were trained in pairs: In each trial, one bat ('observer') had to observe and remember the flight-trajectory of the other bat ('demonstrator'). After a short delay, the observer had to imitate the demonstrator and fly along the same flight-trajectory to receive a reward- a spatial delayed match-to-sample task, which required the observer to pay close attention to the demonstrator's behavior. We recorded neurons in hippocampal area CA1 of the observer bat during this task, using a tetrode-microdrive and a miniaturized wireless electrophysiology system that allows recording individual neurons during flight. To control for the known spatial

properties of hippocampal place-cells, we did two things: first, the observer hung at a fixed position while observing ('space-clamp'); and second, we used an accelerometer to exclude neural activity due to head-movements. Our preliminary data suggest that some CA1 neurons in the observer's hippocampus represent both the bat's own position (place cells) as well as the position of the conspecific. Further, many of these cells were modulated by the direction of the conspecific's flight - similar to place cells that are modulated by the animal's own flight directionality. Taken together, these data indicate a possible role for the hippocampus in social-spatial cognition.

Disclosures: D.B. Omer: None. **N. Ulanovsky:** None. **L. Las:** None.

Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Institutional Development Award (IDeA) Network for Biomedical Research Excellence from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 2P20GM103430

Title: Enhanced acquisition in reference memory due to social housing

Authors: *V. L. TEMPLER, K. PALFRAMAN;
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Abstract: Studies with both humans and nonhuman animals suggest numerous effects of social living, such as stress and neuroprotective benefits, both of which may affect cognitive ability. The aim of this study was to determine the cognitive and behavioral impact of social vs. individual housing in rats. In order to best model disparate levels of sociality in human populations, 20 male Long-Evans rats were housed in enriched cages and were divided into two groups: 10 socially housed (SH) rats lived together in a large cage, and 10 non-socially housed (NSH) rats lived in individual cages where they could see, smell, and hear other rats but had no physical contact with them. These housing conditions were maintained throughout development

and into adulthood, at which point working memory, reference memory, and long-term memory were assessed in spatial contexts using multiple renditions of the Barnes Maze (BM) and Radial Arm Maze (RAM). SH rats displayed improved acquisition in reference memory, but not long-term memory performance, as compared to NSH rats in both the BM and RAM. At the end of acquisition in the RAM, when reference memory errors and exploratory behavior was equalized between groups, the SH animals also showed improved working memory as demonstrated in decreased working memory errors. However, after reference memory for the RAM was learned and over-exploratory behavior in SH rats was extinguished, group differences in both reference memory and working memory errors across groups disappeared. In order to further control for differences in locomotor behavior and examine overall levels of anxiety, subjects were also tested on the Elevated Zero Maze and Open Field Test, which revealed no group differences. We conclude that SH rats show enhanced learning in spatial memory tasks with increased opportunities to learn correct locations.

Disclosures: V.L. Templer: None. **K. Palframan:** None.

Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

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Program#/Poster#: 553.11/LLL32

Topic: H.01. Animal Cognition and Behavior

Title: Central cholinergic function: impact of limited environmental enrichment on spatial learning and memory deficits in the rat

Authors: *J. MANOR[1,2], M. L. PARMENTER[2], E. P. WIERTELAK[2,1];
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Abstract: Alzheimer's disease is a neurodegenerative disease characterized by severe memory impairment and progressive deterioration in cognitive function. Research indicates that the blockade of central muscarinic ACh receptors results in learning and memory dysfunction. Scopolamine is an anticholinergic drug that targets and antagonizes muscarinic ACh receptors, which mirrors the aforementioned cognitive dysfunction and dementia symptoms. Studies have demonstrated that while scopolamine impairs memory and learning, environmental enrichment (EE) can reverse the learning and memory deficits. The present study examined the effects of one hour per day of EE on improving spatial learning and memory in rats, thereby reversing the anticholinergic effects of scopolamine. Subjects were grouped into a standard or enriched condition for the EE training. All subjects were housed in standard cages for three weeks, and the enriched group was removed once per day to receive one hour of play in an enriched

environment with four other conspecifics. All animals were assessed using the Morris water maze spatial navigation task (MWM). Half of the subjects in the enriched and standard condition were injected with scopolamine (0.6 mg/kg, s.c.) thirty minutes prior to testing in the MWM, and the other half of the subjects were injected with saline solution (vehicle control). Results indicate that subjects with one hour of enrichment per day showed significantly decreased latencies to reach a hidden platform compared to those in the standard condition for both drug conditions. These data provide evidence that environmental enrichment has the ability to improve cognitive functioning and potentially, may ameliorate dementia symptomology, by countering ACh deficits. Future research will examine more directly the effect EE may have on cholinergic circuitry.

Disclosures: J. Manor: None. **M.L. Parmenter:** None. **E.P. Wiertelak:** None.

Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

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Topic: H.01. Animal Cognition and Behavior

Support: Japanese Government: 26330296 - Grants-in-Aid for Scientific Research 2014~2016

Title: Investigation of the relation between rat`s navigation strategy and brain activity

Authors: *E. RAMA[1], G. CAPI[4], M. JINDAI[2], Y. FUJIMURA[1], N. TANAKA[3], S. KAWAHARA[3];
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Abstract: Robot self localization and goal directed navigation in a complex environment are still challenging tasks. It has been well known that rats accurately and rapidly navigate in a complex space by localizing themselves in reference to the surrounding environmental cues. As the first step to incorporate the rat's navigation strategy into the robot control, we analyze the rats' strategies while it navigates in a multiple Y-maze. In the present study, we analyzed the effects of distal and remote cues on rats navigation behavior by changing the lighting condition. The multiple Y-maze has a tree-like structure made of a four Y-junctions. Therefore, the rat has to follow the appropriate route to reach the target food location. During training each rat was placed on the starting position and allowed to navigate freely on the maze environment until it reaches the target food position, which was fixed throughout the experiment. To help the rat to localize

itself and to memorize the target route, in the surrounding walls were placed pictures of a different signs (distal cues). First the rats were trained to learn the maze in a lighted environment until they reached an asymptotic level of learning. Then the learned behavior was tested in dark environments. The learning performance was evaluated by percentage rate of selecting the correct route, time to reach the food location and running speed. We found that during the learning phase in a bright condition rats showed different navigation behavior. Some of the rats learned the task quickly and showed a stable performance, while other rats required more time to learn and showed an unstable behavior. When we performed the experiments in dark environment rats which learned the task faster, adapt also quickly with the new environment condition. Based on the behavior results we suggest that rats might use two different navigation strategies to solve the multiple Y-maze task: one dependent on the distal visual information and the other on the local or internal information. This is consistent with many other studies so far, which have demonstrated that hippocampus plays an important role in processing and/or storing spatial memory using distal cues while the striatum is involved in selecting the sequence of actions based on the internal and local cues. In order to verify our hypothesis, the same experiments, with recording electrodes for local field potential (LFP) in these brain regions, are performed. Now, the analysis of the LFP taken from the rat's brain is in progress. The preliminary results show interesting relation between recorded LFP and rats' navigation strategy.

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Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

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Title: Training recurrent networks for neural solutions to realistic navigation tasks

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Abstract: Sensory noise and ambiguous cues make self-localization during navigation computationally challenging: Path integration causes location estimates to deteriorate quickly and landmarks are often spatially extended (e.g. walls) or look similar to other landmarks, thus providing only partial position information. Worse, in novel environments, landmark positions are unknown and must be learned while navigating. How brains perform these difficult computations is largely unknown. Engineering solutions require sophisticated probabilistic algorithms based on particle filters that update several hypotheses simultaneously over time, but are hard to map to biological neurons.
We define several problems that crystallize typical navigation challenges: Self-localization in a circular 1D environment with several indistinguishable landmarks, known circular and polygon-shaped 2D environments with extended, featureless boundary walls, and novel 2D polygon-shaped environments whose boundaries have to be learned during navigation. We take a model-free approach and generate neurally plausible solutions by training recurrent networks with hidden layers, then scrutinize their performance, errors, and dynamics.
The networks learn to update their estimates through velocity integration, integrate landmark information, and use memory of the last landmark encounter to choose between competing location hypotheses. The network performance matches the optimal particle filter (known environments) and substantially outperforms pure path integration (unknown environments), evidence that it can learn new maps. Reminiscent of remapping, the hidden units dynamically switch their tuning to code for the relevant statistics at a given time, such as location relative to the last observed landmark or to absolute location. These results demonstrate that hard navigation tasks can be solved by deterministic networks and provide predictions for neural representations during real-world navigational challenges.

Disclosures: I. Kanitscheider: None. **I.R. Fiete:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: German Research Foundation (DFG): FOR2143

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Title: Deep brain two-photon calcium imaging of dentate gyrus granule cells in awake mice

Authors: *T. HAINMÜLLER, M. BARTOS;
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Abstract: The hippocampus is an essential brain center for the acquisition of conscious memories. Formation of functional neuronal ensembles in the dentate gyrus (DG), the input region to the hippocampus, is a critical step in the genesis of episodic memories (Denny et al., Neuron, 2014). Furthermore, activation of these ensembles can be used to manipulate existing memories in mice or to craft artificial ones (Liu et al., Nature, 2012; Ramirez et al., Science, 2013). In spite of this important function, the knowledge on how these neuronal groups emerge during memory acquisition is scarce.
The study of neurons in the DG is hindered by two factors: First, the activity of granule cells (GCs), the principal neurons of the DG, is extremely sparse, with most neurons being silent throughout behavioral recording sessions (Pernía-Andrade & Jonas, Neuron, 2014). Therefore, a large number of GCs must be observed simultaneously in order to record activity of the few coactive cells that form a functional memory ensemble. This is hard to achieve using standard electrophysiological recording techniques like tetrode-recordings. Second, the DG is located deep within the brain and therefore difficult to access for many of the current standard recording methods in neuroscience, such as patch-clamp recordings or two-photon imaging approaches.
To overcome these technical obstacles and record simultaneously from a large number of DG neurons, we have developed a novel approach to perform two-photon calcium imaging in the DG of awake, head-fixed mice performing behaviors in a virtual-reality environment (Dombeck et al., Nature Neurosci., 2010). We were able to simultaneously record the activities of a subset of DG neurons, including neurochemically identified cell types labelled with the fluorescent protein tdTomato. Our recordings revealed location related activity (‘place fields’) in some of these cells, including hilar non-GC neurons. In summary, our approach will enable the possibility to investigate the formation of functional DG neuron ensembles and their interactions with other cell types in the DG during memory related behaviors in a virtual environment.

Disclosures: T. Hainmüller: None. **M. Bartos:** None.

Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

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Topic: H.01. Animal Cognition and Behavior

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2014-MSCRFF- 0711

Title: Optogenetic identification of adult-born granule cells in freely behaving mice

Authors: *S. KIM[1,2], W. HUANG[1,2], D. GOODSMITH[3], K.-J. YOON[1,2], K. M. CHRISTIAN[1,2], J. J. KNIERIM[3,4], G.-L. MING[1,2,3], H. SONG[1,2,3];
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Abstract: Constitutive neurogenesis occurs in the dentate gyrus (DG) of the mammalian hippocampus throughout life. Newborn granule cells integrate into local hippocampal circuitry and have been suggested to function in hippocampus-dependent learning, memory and affective behaviors. Immature granule cells show increased excitability and plasticity in acute hippocampal slice recordings, suggesting that these cells may be more active than mature granule cells. However, the electrophysiological properties of newborn neurons in vivo are not well-characterized due to the technical challenges of identifying the age of putative granule cells during extracellular single unit recordings. To identify and record newborn neurons in the intact brain, we established a novel inducible transgenic mice model to express the light-activated opsin channel, channelrhodopsin (ChR2), in a birthdated cohort of newborn neurons. Five days of systemic tamoxifen injections in Tbr2-CreERT2 mice selectively targets intermediate neural progenitors in the adult mouse hippocampus. Using this Tbr2-mediated genetic targeting strategy, ChR2 was systematically expressed in a highly specific and robust manner (i.e. approximately 1000 ChR2-YFP+ cells per dentate gyrus). At two weeks post tamoxifen injection, 99% of YFP+ cells expressed doublecortin (a marker of immature neurons). By combining optogenetics with in vivo extracellular recording, we identified several light-responsive units, with less than 5 msec latency after the onset of the light stimulation. We are currently optimizing the conditions to test the hypothesis that immature granule cells are more active than mature granule cells during exploratory behavior. Recording sessions in multiple distinct environments will allow the measurement of spatial selectivity and remapping properties of both immature and mature granule cells.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: R01 NS039456

Johns Hopkins University Brain Science Institute

Title: Distinct spatial firing and remapping properties of cells in the granule cell layer and hilus of the dentate gyrus

Authors: *D. GOODSMITH[1], K. M. CHRISTIAN[2,3], S. KIM[2,3], H. SONG[2,3,4], J. J. KNIERIM[1,4];
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Abstract: The dentate gyrus (DG) was traditionally thought to perform pattern separation due to the projection of overlapping entorhinal inputs onto a larger, sparsely firing granule cell (GC) population, leading to the recruitment of independent ensembles of active GCs in response to small changes in input (Marr, 1969; McNaughton & Nadel, 1990). Although GCs make up the majority of cells in the DG, the hilus contains a number of other cell types, including excitatory mossy cells. Recent evidence suggests that DG cells with single or multiple firing fields correspond to different cell populations (Neunuebel & Knierim, 2012). It is unclear whether this heterogeneity in spatial firing properties can be attributed to anatomically distinct populations in the granule cell layer (GCL) and hilus.

We recorded from the DG and CA3 of 8 rats while they foraged for food in four distinct environments. Based on the baseline firing properties of previously reported cells recorded from tetrodes located unambiguously in the hilus or GCL (23 and 37 cells respectively), together with an additional 31 cells recorded in CA3c, we trained a random forest classifier to identify putative GCL, hilus, and CA3 cells from a larger data set (245 cells total). Consistent with our previous results, putative GCs (n=112) were rarely active (5% of cells per session), and the majority of these cells had single fields, while putative hilus neurons (n=35) tended to have multiple fields and >75% had fields in ≥ 3 environments. The firing of active cells in CA3 (n=98) and the GCL was similar (single field, single room), but a higher percentage (23%) of CA3 cells was active in a given session.

The overlap of firing rates between environments (defined as the product of normalized mean firing rates) was higher than a shuffled distribution for cells in the hilus, but not GCL, suggesting that different populations of putative GCs were active in each environment. To test remapping in the GCL and hilus populations, we next generated population vectors (PV) of co-recorded cells in the hilus or GCL. In both the hilus and GCL, the maximum correlation between pairs of environments (after shifting and/or rotating one PV) never exceeded the 99th percentile of a shuffled distribution. Together, these results suggest that cells in both the GCL and hilus can support DG pattern separation through two distinct mechanisms. Consistent with Marr's model of "expansion recoding," unique populations of sparsely firing GCs appear to be recruited in each environment. In contrast, hilus cells may encode distinct environments through changes in the spatially correlated firing of the entire active population (Leutgeb et al., 2007).

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Poster

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R01 NS039456

Title: CA1 place fields respect local surface texture boundaries

Authors: ***C.-H. WANG**[1], J. D. MONACO[2], G. RAO[1], S. S. DESHMUKH[3], J. J. KNIERIM[1]; [1]Krieger Mind Brain Inst., [2]Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [3]Ctr. for Neurosci., Indian Inst. of Sci., Bangalore, India

Abstract: Emerging evidence shows that local cues exert considerable influence over place-cell firing. For example, “landmark-vector cells” develop place fields located at a similar distance and direction from objects placed on the apparatus (Deshmukh and Knierim, 2013). Systematic manipulations of local surface cues lead to corresponding place field rotation or remapping (Shapiro *et al.*, 1997; Brown and Skaggs, 2002; Knierim *et al.*, 2002). In these studies, place cells were recorded as rats explored an apparatus with different sections covered by different textured surfaces. Some of the place fields followed the local surface cues when the local and global cue sets were rotated in opposite directions. Although the remapping and rotation properties of place fields have been widely examined with manipulations of surface cues, few studies have focused on the properties of individual place fields at the transition boundaries between different surface cues and the distribution of place fields with respect to the boundaries. Given the salience of a sudden change of surface texture, we hypothesized that the transition boundaries could be salient cues that influence the precise firing locations of place cells.
In the present study, we demonstrate that CA1 place fields tended to terminate near texture boundaries when the rats foraged for food on a circular track. The track width was 10 cm and its outer diameter was 76 cm. Each quadrant of the circular track was covered by a different texture surface with distinct tactile and visual cues, and salient global visual cues were present at the perimeter of the circular, curtained environment, approximately 1.35 m from the track. We analyzed 2619 place fields from the CA1 region of 27 rats, from a database composed of both published and unpublished data. Examples of individual place fields were observed that fired

specifically at a texture boundary, in the middle of a texture patch, or that fired across two different texture patches. However, when the entire sample was analyzed, the place field boundaries (i.e., the onset and offset of the place field firing) were not uniformly distributed on the track (Kolmogorov-Smirnov test, $p < 0.0001$). Rather, the boundaries tended to coincide with the texture boundaries (chi-squared test, $p < 0.0001$; bootstrap, $p < 0.0001$). These results show that the CA1 spatial representation respects the boundaries between different textured surfaces. This phenomenon may enable segmentation/compartmentalization of experiences by natural environmental boundaries.

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Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

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Johns Hopkins University Discovery Award

Title: Extreme control of CA1 spatial maps by coherently moving virtual reality landmarks

Authors: ***R. P. JAYAKUMAR**[1], M. S. MADHAV[2], F. SAVELLI[2], M. BREAULT[3], H. T. BLAIR[5], J. J. KNIERIM[2], N. J. COWAN[4];
[2]Zanvyl Krieger Mind/Brain Inst., [3]Biomed. Engin., [4]Mechanical Engin., [1]Johns Hopkins Univ., Baltimore, MD; [5]Dept. of Psychology, UCLA, Los Angeles, CA

Abstract: Constructing a cognitive map such as that expressed by place cells in the hippocampus requires the integration of self-motion cues and landmark information that can stabilize the map with respect to the external world. Using a planetarium style virtual reality (VR) dome, we investigated the dynamics of the CA1 hippocampal place cell network by coherently manipulating distal landmarks, putting them in conflict with integrated self motion cues. In the VR dome, rats ran in a circular path experiencing unaltered inertial and proprioceptive feedback. The surrounding visual scene consisted of salient virtual landmarks that were either static or rotated coherently as a function of the rat's running speed. The rotation resulted in the continuous movement of the landmark constellation against the laboratory (absolute) reference

frame.
Previously (Madhav et al., 2015, SFN Abstracts), we showed anecdotally that the place cell map was locked to the landmarks at relative (to the landmarks) velocities ranging from close to zero (i.e., producing a treadmill-like experience) to three times the actual running speed of the animal. Here we quantified the influence of the moving landmarks on the place cell network using two independent, population-level approaches: a position decoder based on unsorted spikes, and spatial frequency estimation scheme based on Fourier analysis of firing rates. Using unsorted spikes when the landmarks were held stationary, we trained a position decoder (Kloosterman et al., 2013). The decoder was then applied to epochs where the landmarks were moving. When the landmark constellation was moving, the decoded position tracked the rat's position relative to the landmark constellation (not the static laboratory reference frame). The tracking error increased for higher landmark velocities, which may be attributed to an increasing conflict between self-motion cues and landmark cues, the degrading of the decoded position due to partial remapping of the place fields, or a combination of the two. To address this ambiguity, we developed a Fourier-based algorithm which estimates how the spatial frequency of the place cell population evolves during an experiment. This analysis is relatively insensitive to remapping. In all cases, the decoded spatial frequency was dramatically more compatible with a map controlled by the virtual landmarks than by the laboratory reference frame. These results show that visual landmarks can override self-motion cues even under extreme conditions of conflict between self-motion and landmark information.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: R01 MH094146

R01 NS039456

Title: Lateral entorhinal cortex neurons are sensitive to circular movement direction relative to the center of an open-field arena

Authors: ***C. WANG**[1,2], H. LEE[1,2], S. DESHMUKH[3], G. RAO[1,2], D. YOGANARASIMHA[4], F. SAVELLI[1,2], J. J. KNIERIM[1,2];

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Abstract: The lateral entorhinal cortex (LEC) is hypothesized to provide the hippocampus with rich multisensory input that represents the items and events of an episodic memory. Compared to the medial entorhinal cortex (MEC), little is known about the behavioral correlates of LEC neuronal activity. LEC neurons convey much less spatial information to the hippocampus than the MEC. Instead, LEC neurons appear to encode more information about the objects present in an environment, including information about the remembered locations of previously present objects. In the presence of objects, a small number of LEC neurons appear to have spatially tuned "place fields" away from the objects. Thus, although a number of LEC neurons have activity that can be classified as spatial/object correlates, the influence of other behavioral variables remain open to investigation. We recorded LEC neurons from 15 rats as they foraged for food in a large, open-field arena (8 rats in a rectangular arena with objects and 7 rats in a square arena with no objects). Surprisingly, we found that approximately half (235/447) of LEC cells were significantly modulated by whether the rat was moving in a clockwise or counterclockwise direction relative to the center of the behavioral arena. Some cells fired almost exclusively when the rat was running in the clockwise direction, whereas simultaneously recorded cells fired almost exclusively when the rat was running in the counterclockwise direction. Although the underlying mechanism for this circular direction selectivity is unknown, one intriguing possibility suggested by this phenomenon is that LEC neurons are influenced by sensorimotor events happening during movement in the arena.

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Poster

553. Cortical and Hippocampal Circuits: Spatial Navigation

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Topic: H.01. Animal Cognition and Behavior

Title: A simple relational task produces a higher immediate early gene expression in the avian hippocampus

Authors: *M. J. ACERBO[1], J. RICK[2], K. PANFIL[2], A. KOSKI[2], M. STACHO[3], O. GÜNTÜRKÜN[3], O. LAZAREVA[2];
[1]Psychology, Iowa State Univ., Ames, IA; [2]Psychology, Drake Univ., Des Moines, IA; [3]Psychology, Ruhr-University Bochum, Bochum, Germany

Abstract: Recently, we showed that hippocampal lesions in pigeons selectively impair relational behavior in a transitive inference task. In the current study, we have explored the involvement of the hippocampus in another traditional relational task, transposition. Unlike transitive inference, transposition does not require manipulating relational information; instead, animals are simply trained to learn the physical relation between the stimuli (e.g., larger or smaller) and use the same relation when presented with novel pairs of stimuli. We trained two groups of pigeons to complete a task either relationally or associatively. The pigeons learned to discriminate two pairs of circles of different sizes, S1-S2 and S3-S4 (where S1 was the smallest and S4 was the largest). For the relational group the smaller (or the larger) circle in each pair was rewarded. For the associative group the smaller circle in one pair and a larger circle in another pair were rewarded; these pigeons had to memorize each exact stimulus instead of relying on their relationship. Training was followed by a test with two novel pairs, S2-S8 and S2-S3. Next, we analyzed c-Fos and ZENK expression in the NCL and four hippocampal areas: dorsomedial ventral (DMv), an area similar to the mammalian CA1; dorsalmedial dorsal (DMd), an area similar to the mammalian CA3; and dorsolateral ventral (DLd) and dorsolateral dorsal (DLd), areas similar to the mammalian entorhinal cortex. We found greater number of cells expressing c-Fos in the relational group than in the associative group for all four areas, although only the DLd activity was significantly higher. Similarly, we found greater number of cells expressing ZENK for all four areas in the relational group than in the absolute group. However, statistical differences were restricted to the DLd area of the right hemisphere. These results indicate that avian hippocampus may be involved in relational tasks that are based on a physical relationship and that do not explicitly require encoding or manipulating relationships between different training stimuli. Furthermore, our results imply that simple relational tasks may share a common substrate with spatial learning.

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Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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Alberta Innovates Health Solutions Fellowship

MH099682

AG049090

Alberta Innovates Health Solutions Graduate Studentship

Title: A methodological pipeline for serial-section imaging and tissue realignment for assessment of whole brain connections, functional activation and markers of disease

Authors: *L. MESINA[1], A. A. WILBER[2,1], B. J. CLARK[3], S. DUBE[1], A. J. DEMECHA[1], M. ECKERT[1], C. E. L. STARK[2], B. L. MCNAUGHTON[2,1];
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Abstract: Understanding the neurobiological basis of cognition and behavior, and disruptions to these processes following injury and disease, requires a large-scale assessment of neural populations, and knowledge of their patterns of connectivity. We present an analysis platform for large-scale investigation of functional and neuroanatomical connectivity in rodents. Retrograde tracers were injected (for details see Wilber et al 2015 Frontiers in Neural Circuits) and in a subset of animals behavioral tests to drive immediate-early gene expression were administered. This approach allows users to perform whole-brain assessment of function and connection in a semi-automated quantitative manner (for details see Mesina et al 2016 Journal of Neuroscience Methods). Brains were cut in the coronal plane, and an image of the block face was acquired. Wide-field fluorescent scans of whole sections were acquired and analyzed using a Matlab toolkit, available for download at https://github.com/lilicamd/BrainSegmenterGUI. The platform utilized open-source and custom software to accommodate a largely automated analysis pipeline in which neuronal boundaries are automatically segmented, the positions of segmented neurons are co-registered with a corresponding image acquired during sectioning, and a 3-D representation of neural tracer (and other products) throughout the entire brain is generated (results available at https://github.com/lilicamd/MethodsSpace). Our focus on segmented units of interest (e.g., NeuN labeled neurons) and restricting measures to these units produces a flexible platform for a variety of whole brain analyses (measuring activation, connectivity, markers of disease, etc.). This open-source toolkit allows an investigator to visualize and quantify whole brain data in 3-D, and additionally provides a framework that can be rapidly integrated with user-specific analyses and methodologies.

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Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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NS20331

Title: Directionality in distal CA1 is driven by sensory cues, while proximal CA1 is influenced by self-motion differences

Authors: *Z. NAVRATILOVA[1], L. T. HOANG[2], J. L. VALDES[3], M. TATSUNO[1], B. L. MCNAUGHTON[1];
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Abstract: The primary correlate of hippocampal firing is location in space (O'Keefe and Dostrovsky 1971). Other factors can influence the firing rates of CA1 and CA3 'place cells' (e.g. Leutgeb et al. 2005), the strongest of which seems to be running direction during bi-directional traversals of narrow tracks (McNaughton et al. 1983). We recently showed that these directional differences are not present during the first traversals of the track, suggesting that they are not due to deficient path integration (the ability to sum up distances and directions traveled to calculate relative position), but instead are due to learning different associations to the traversals of each direction (such as sensory cues; Navratilova et al. 2012).
CA1 and CA3 place cells receive information about location and self-motion from neurons in the medial entorhinal cortex (MEC), and sensory information from the lateral entorhinal cortex (LEC). While CA3 cells each receive inputs from both entorhinal cortices, cells in the proximal (to CA3) half of CA1 receive inputs from MEC, and the distal half of CA1 is innervated by LEC axons. All CA1 cells also receive inputs from CA3, and all show location-specific firing, with distal CA1 cells expressing a larger number of place fields (Henriksen et al., 2010). Larger numbers of distal CA1 cells are recruited during a non-spatial memory task (Nakamura et al., 2013).
We hypothesized that distal CA1 cells are more influenced by sensory inputs, and thus more likely to develop directionality in an environment dominated by distal visual cues. We recorded from 12 tetrodes along the proximal-distal axis of CA1 while rats ran back and forth between two food dishes. One half of the track was rich in local sensory cues, and the other half had no local cues. Distant visual cues were visible. Cells from both proximal and distal CA1 showed differences in firing rates between running directions after several traverses of the track. However, the population of proximal CA1 cells showed a high correlation between traverses of

the track in opposite directions, regardless of the type of cue that was present, while the distal CA1 population showed a low correlation between opposite directions when only distant visual cues were present. As the rats ran the track repeatedly, they developed stereotyped behavior, including running faster when approaching a food dish, and slower when leaving one. This difference in running speed in opposite directions significantly influenced the firing rates of proximal (but not distal) CA1 cells. Thus, firing rate differences between opposite direction traverses were due to running speed differences in proximal CA1, and visual cue differences in distal CA1.

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Poster

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MH4682316

Alberta Innovates Health Solutions Fellowship

Title: Emergence of sequential multineuronal reactivation in rat parietal cortex in parallel with learning

Authors: *I. SKELIN[1,3], A. A. WILBER[3,2], C. J. MONTZ[3], S. KILIANSKI[3], B. MCNAUGHTON[3,2];
[1]Neurosci., [2]Univ. of Lethbridge, Lethbridge, AB, Canada; [3]Univ. of California Irvine, Irvine, CA

Abstract: Tuning to specific combinations of linear, and angular velocity is organized in a modular fashion in rat parietal cortex, as assessed by pooling multineuronal spiking activity (MUA) recorded on multiple single tetrodes. Based on this modularity, we sought to determine if patterns of module activation that are apparent during behavior undergo reactivation during post-experience sleep - a postulated mechanism of memory consolidation previously demonstrated on single neuron level (Wilson and McNaughton, 1994). We found evidence for modular reactivation using both cross-correlation of MUA patterns between tetrode pairs and explained variance, methods which are sensitive to correlated activity at the whole epoch level. To test the

hypothesis that sequential module activity patterns would be reactivated during post-experience sleep, we applied the *template matching* method (Euston et al., 2007). Templates were created by multiple trial averaging of either the total spiking activity from a single tetrode (module) or the envelope amplitude of Hilbert-transformed high-frequency (300-900 Hz) component of the local field potential (LFP), recorded from a single wire on the same tetrode. The time-windows for template construction were either 1 sec preceding ('approach') or 1 sec following the brain stimulation reward ('depart'). The Pearson correlation coefficient was calculated between the templates and the sliding window of time-compressed (5x) spiking or Hilbert envelope activity during sleep. The significance of matching was assessed by comparing the correlation coefficient obtained using behavioral to100 shuffled templates. High matching values are concentrated around the times of hippocampal sharp wave/ripples (+- 200 ms), suggesting that populations of modules reactivate with hippocampal populations. The ripple-triggered reactivation peaks tend to emerge and get higher following repeated behavioral training sessions, even when the number of trials decreases, and independent of the number of ripples during sleep. The templates constructed based on the Hilbert-transform envelope of high frequency power show similar dynamics, but appear more sensitive than templates based on multi-neuronal spiking activity. These results support the conclusion, that memory trace reactivation and hippocampal-cortical interactions can be observed at the level of modular activity, expressed either as multi-neuron spiking or high frequency LFP power fluctuations, and indicate that these processes can potentially be studied without resort to recording from many individual isolated units.

Disclosures: I. Skelin: None. **A.A. Wilber:** None. **C.J. Montz:** None. **S. Kilianski:** None. **B. McNaughton:** None.

Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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AG049090

Alberta Innovates Health Solutions Fellowship

Title: Modular memory reactivation in the parietal cortex

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Abstract: A fundamental framework for neural coding in the parietal cortex (PC) is egocentric (e.g., body-centered, route-centered) and a preponderance of evidence suggests neural coding within this framework by single cells in PC (e.g., Zipser and Andersen, 1988; McNaughton et al., 1994; Nitz, 2006; Whitlock et al., 2012; Wilber et al., 2014). However, studies describing the larger organizational structure of these single cells (e.g., large scale populations of cells) have been thus far confined to theoretical or computational approaches (e.g., McNaughton et al., 1995; Xing and Andersen, 2000; Byrne, Becker, and Burgess, 2007). Therefore, we looked for evidence of population level encoding in egocentric coordinates in the rat PC. To do this we assessed pooled multi-neuronal spiking activity (MUA) recorded on single tetrodes as a function of egocentric cue light location, linear, and angular velocity in rats that had been trained to either run 1) a cued random spatial sequence to 32 light locations evenly spaced around the perimeter of a circular platform or 2) a complex repeating element spatial sequence from memory. We found that the MUA recorded on a single tetrode were frequently significantly tuned to a specific self-motion state and often anticipated that movement. Next, we set out to test the hypothesis that these cortical modules would participate in memory reactivation during rest. As a first step, we looked for evidence of memory replay using two methods: explained variance (EV) and cross-correlations. First, we utilized EV performed on the MUA from each of up to 18 tetrodes to look for sessions where replay appeared to be strong. This method utilizes a partial correlation between the *task* & *post-task rest* session after controlling for correlations between the *pre-task rest & task*. Next, for data sets in which EV suggested replay was likely to occur across the PC modules, we assessed memory replay using cross-correlations between the population of module pairs (similar to Euston et al., 2007). For this analysis, PC module pairs with significant cross-correlations are identified. After normalizing, cross-correlations are then sorted, and plotted for *pre-task-rest*, *task* and *post-task-rest*. We found that, for data sets in which EV indicated that memory reactivation was likely to occur, cross-correlations suggested that co-activation between module pairs was more likely in *post-task-rest* for modules that were strongly co-active during behavior. Thus, we have shown modular coding of movement. Further, interactions between modules during *post-task-rest* suggest that inter-modular interactions play a role in normal learning and memory.

Disclosures: A.A. Wilber: None. **I. Skelin:** None. **C.J. Montz:** None. **S. Kilianski:** None. **B. McNaughton:** None.

Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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Topic: H.01. Animal Cognition and Behavior

Title: Hippocampal and posterior parietal neural dynamics during two working-memory tasks.

Authors: *I. TAXIDIS, K. SAMADIAN, E. HOFFBERG, A. MYLAVARAPU, J. SADIK, N. R. SABOORI, M. BEDROSSIAN, P. GOLSHANI;
UCLA, Los Angeles, CA

Abstract: Various studies suggest that both hippocampus and posterior parietal cortex (PPC) are activated during delayed-response, working-memory behavioral tasks. Spiking sequences have been observed in CA1 during the delay in a match-to-sample olfactory task [1] and a spatial alteration task [2] in head-fixed and freely-moving rats respectively. Similar sequences were found in the mouse PPC during a visually-cued virtual-navigation task [3]. These sequences may be critical for task performance, but their link to working-memory is not well understood. Are they associated with the actual memory of the stimulus, irrelevantly of the stimulus type, or could they encode preparatory activity for the ensuing post-delay response? How do these sequences respond when the delay is extended or reduced? To gain more insight into these questions, we have trained head-fixed mice to perform two olfactory working-memory tasks. A delayed odor-discrimination lick left/right (DOD) and a delayed non-match-to-sample link/no-lick task (DNMS). In DOD, stimulus-memory cannot be dissociated from preparatory activity for the response, whereas in DNMS, only stimulus-memory is relevant during the delay. Using *in vivo* two-photon microscopy in PPC, during the DOD task, we recorded from hundreds of neurons over multiple days, with varying delay periods. We observed task-modulated activity in several neurons that discriminated between left/right trials or trial stages, leading to spiking sequences covering the entire trial duration. These neurons typically retained their task modulation during variable delays. Interestingly, lesioning the PPC with bilateral aspirations did not affect the performance of well-trained animals in either task, suggesting that PPC sequences are not essential for working-memory in a non-navigational olfactory task. We are extending two-photon imaging to the dorsal CA1, during the DNMS task, to examine the involvement of the hippocampus. Understanding whether and how the posterior parietal cortex and hippocampus can generate sequential activity, and how this activity is linked to the memory load are critical steps for understanding the mechanisms underlying working memory.
1. MacDonald et al., Distinct hippocampal time cell sequences represent odor memories in immobilized rats. *J Neurosci*, 2013.
2. Pastalkova et al. Internally generated cell assembly sequences in the rat hippocampus. *Science*, 2008.

3. Harvey et al. Choice-specific sequences in parietal cortex during a virtual-navigation decision task. *Nature*, 2012.

Disclosures: **I. Taxidis:** None. **K. Samadian:** None. **E. Hoffberg:** None. **A. Mylavarapu:** None. **J. Sadik:** None. **N.R. Saboori:** None. **M. Bedrossian:** None. **P. Golshani:** None.

Poster

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Title: Movements in the vertical dimension organize the timing of spatially-specific firing in the hippocampus and posterior parietal cortex

Authors: ***L. E. SHELLEY**, D. A. NITZ;
UCSD, La Jolla, CA

Abstract: Neurons of both the hippocampus and posterior parietal cortex map the position of an animal in external frames of reference. Hippocampal neurons primarily map position within the space of the observable environment. In a complementary fashion, posterior parietal cortex neurons map position within the space of known routes irrespective of the position of those routes in the observable environment. Maps of route position given by the activity of posterior parietal cortex (PPC) neurons appear to derive initially from the tendency of a subset of these neurons to have activity correlates to specific locomotor behaviors such as left or right turns or simple forward locomotion. A mapping of route position is then achieved when robust and reliable modulation in the firing activity during performance of the preferred locomotor behavior occurs as a function of the location of that behavior along a full route.
Recently published experiments suggest that the form by which vertical space is mapped by hippocampal place cells and entorhinal cortex grid cells is fundamentally different and of lower resolution than that of horizontal space. However, the way that an animal moves through a position (e.g., the overall trajectory taken and/or the individual actions executed) can potently modulate spatially-specific firing in both hippocampus and PPC. As movement in the vertical dimension involves distinct types of action and trajectories, we addressed the possibility that posterior parietal cortex might encode specific actions associated with movement in the vertical dimension and that hippocampal neurons' place specific firing would be altered by such actions. Single neuron recordings in both regions were collected as animals made traversals of a long, 5-

looped, squared spiral track. The animal's route had vertical components in the form of stairways and ramps. Based on position tracking data, the beginnings and endings of individual stair-stepping movements were identified. Analyses of firing relative to the phase of these movements revealed that such actions impact the activity of both hippocampal and PPC neurons. Such firing included the tendency for a sub-population of hippocampal place cells to exhibit greater place-specific firing over specific phases of the hop to landing cycles associated with stairway climbing. Together, the findings identify a significant new form of action encoding in PPC and a new form of temporal organization in hippocampal neurons' place-specific firing. The latter finding suggests a temporal signature for movements in the vertical dimension that could be incorporated in the generation of episodic memories.

Disclosures: L.E. Shelley: None. **D.A. Nitz:** None.

Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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NSF IOS-1149718

Title: Hippocampal and Posterior Parietal Cortex spatial encoding during pursuit.

Authors: *A. S. ALEXANDER[1], A. M. CONNER[2], J. C. TUNG[2], D. A. NITZ[2];
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Abstract: ***Aims:*** Known forms of spatial representations, such as 'place cells' of the hippocampus (HPC) and 'route cells' of the posterior parietal cortex (PPC) are utilized for navigation and memory. Neurons that exhibit these spatial firing properties are commonly examined during free foraging or route running along tracks. These tasks have elucidated much about the encoding of spatial relationships. However, animals are capable of more complex navigational feats than these paradigms probe. In the wild, rats will travel in packs and hunt small prey. These behaviors involve pursuit, a form of flexible navigation wherein the goal is to track a target in real time. Navigation of this type requires quick decision making to coordinate the musculature, but also often requires the animal to track it's movements within the broader environment to return to a home nest. It is currently unknown how the aforementioned brain regions are recruited during pursuit. ***Methods:*** We developed a novel pursuit paradigm wherein

rats must catch a target moving in pseudorandom trajectories within an environment for reward. At fixed environmental locations, the moving target performed a stereotyped trajectory that the animal learned and subsequently executed shortcut trajectories, a form of spatial insight. **Results/Conclusions:** We report that PPC neurons generate representations of the stereotyped trajectory, despite the path being embedded within an unpredictable navigational environment. Furthermore, HPC place cells were modulated during pursuit, suggesting that the animal's goals and behavioral context may shape spatial encoding.

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Poster

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Support: Kavli - KIBM #2015-055

NSF - 1149718

Title: Dorsal subiculum encodes functionally analogous path locations

Authors: ***J. M. OLSON**, K. TONGPRASEARTH, E. L. TAO, A. WOOTEN, H. C. LO, D. A. NITZ;
Cognitive Sci., Univ. of California San Diego, LA Jolla, CA

Abstract: Hippocampal neurons often have place-specific firing, but the details of how and when firing fields form and change differs across subregions. These differences in firing field patterns may provide clues to the different functional roles of each subregion. Hippocampus's two main output subregions, CA1 and subiculum, have very different efferent connectivity to surrounding cortices, suggesting they are parts of different functional circuits. Prior work in open field recordings has suggested that CA1 and subiculum may be largely similar, with subiculum neurons tending to have larger firing fields and generalizing more often across environments than CA1. Often in hippocampus, however, the shape of paths the animal takes through an environment impacts the place-specific firing. In the current work, we studied and compared CA1 and subiculum neurons while animals ran on a track with 6 distinct paths - four towards reward locations and two returning to the starting location. The four reward-oriented paths were equal in length, starting at the same segment and consisting of four straight runs interleaved with three 90-degree turns. Two non-overlapping paths of two turns each provided the animal with

options to return to the starting point of the four rewarded routes. All corresponding track segments across the four reward paths are equal length, as are the corresponding track segments of the return paths. This structure results in paths and segments that are spatially separated but functionally analogous in their progression from a choice point towards a goal point. Critically, such sections were parts of different action sequences in addition to being in different positions in the environment. While previous work has shown that CA1 place fields sometimes generalize across spatially local and action-sequence-related locations, preliminary results for CA1 show low numbers of firing fields that are typically not in analogous track locations. However, subiculum neurons often show firing fields in functionally analogous spaces, even in locations in space and routes that involve running in opposing orientations. The findings to date suggest that CA1 and subiculum outputs reflect a difference in information and therefore function to their separate efferents. Consistent with previous reports, more generalization occurs in subiculum, extending to include distinct spatial and behavioral locations that are functionally similar. These results hint to subiculum as a region that could be key to drawing out similarities between experiences and building more abstract spatial relations than typically described in other hippocampal subregions.

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Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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Support: Kavli - KIBM #2015-055

NSF - 1149718

Title: Phase precession in subicular axis cells modulates with field size within individual neurons

Authors: ***K. TONGPRASEARTH**, J. M. OLSON, E. L. TAO, D. A. NITZ;
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Abstract: Phase precession is a well-established organizational phenomenon in place field firing. As an animal moves through a single firing field of a neuron, individual action potentials coincide with earlier phases of the theta band in the local field potential. This phenomenon is

found widely across spatial representations in the hippocampus and related cortices, including CA1, CA3, entorhinal cortex, and subiculum. Across the length of a field, the neural activity precesses through 360 phase degrees, or one cycle, of theta. In recent work, we have recorded from dorsal subiculum while rats navigate a multi-path maze designed for its multiple track segments and travel orientations. The task consisted of four equal length paths of straight runs interleaved with three 90-degree turns that led to different reward sites. Two non-overlapping two-turn return paths then provided the animal routes back to the starting point of the four three-turn routes. Similar to many hippocampal subregions, subicular neurons exhibited altered spatial representations during path-running. Our results from a subpopulation of subiculum neurons (~10%) revealed a novel form of spatial tuning, the encoding of the animal's current axis of travel. These "axis cells" exhibited firing fields in two travel orientations, approximately 180 degrees apart. Here we investigated whether this novel cell type exhibits phase precession. While previous work have reported that phase precession is robust in principal neurons of dorsal subiculum, the same was not observed in head direction cells, the most prominent orientation-specific neurons. Preliminary results provided evidence for robust phase precession, indicating that axis-tuned neurons are likely to be integrated in the temporally organized structure common across the hippocampus and entorhinal cortex. Additionally, a unique feature of our task is the varied length of spatial segments. As a result, field sizes associated with high neural activity in axis cells greatly varied according to the track length of those particular orientations, with lengths ranging from 20-130 cm. As found elsewhere in hippocampus, preliminary results suggested phase precessions through only one cycle of theta for each field of axis cells despite the greatly different firing field sizes, demonstrating a change in phase precession rate within single neurons. This result indicates that these representations are fixed to the distance of each track segment in the two directions and so each segment’s length is built into the subicular representation.

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Poster

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EPSRC grant K015141

Title: Concordant representation of spatial decisions and uncertain environments in hippocampus and visual cortex

Authors: *A. B. SALEEM, J. FOURNIER, K. D. HARRIS, M. CARANDINI;
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Abstract: Most decisions are made based on uncertain information. Multiple models have been proposed for how neuronal populations might represent such decisions and encode uncertainty. One model suggests uncertainty reduces mean rates (Ma et al., 2006). Alternative models include an increased spread of population activity (Zemel et al., 1998), or increased coherent errors (Burak et al, 2009). To test the predictions of these models, we measured population activity in primary visual cortex (V1) and hippocampus (CA1) during spatial decisions in conditions of uncertainty.

We trained four mice to perform a decision task in virtual reality, where we could control sensory uncertainty. The task involved traversing a corridor and licking in a reward zone to obtain a water reward. We modulated uncertainty by setting the visual contrast of cues to low (18% contrast), medium (60%), or high (72%) values. Task performance increased with visual contrast, from 69% correct at low contrast to 85% at high contrast.

Once mice learned the task, we recorded from neural populations in V1 and CA1 simultaneously using multielectrode probes. As expected, features in the environment were encoded by both V1 and CA1 neurons, and we could predict the animal's position from the activity of simultaneously recorded cells from either area using an independent Bayes decoder.

The position estimates decoded from V1 and CA1 were highly correlated with each other. A fraction of these correlations were signal correlations: i.e. they were explained by the fact that activity in both areas depended on position in the corridor. However, we also observed significant noise correlations: if the CA1 population underestimated or overestimated position, the V1 population typically made a similar error.

Sensory uncertainty affected the representation of the environment in V1 and CA1 in distinct ways. In V1, increasing uncertainty reduced mean firing rates, while in CA1 mean firing rates remained unchanged. Instead, population analysis revealed that under high uncertainty CA1 neurons made coherent errors, as expected for an attractor network. The coherent errors made by the hippocampal population were also correlated with behavioural errors made by the animal. Indeed, when the mouse licked outside the rewarded zone, CA1 neurons erroneously placed it in the reward zone.

We conclude that while both V1 and CA1 encode the virtual environment and make correlated errors, the representation of uncertainty is distinct in the two regions: V1 reduces its mean firing rate and CA1 neurons make coherent errors.

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Poster

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Title: Integration of self-motion and visual signals in mouse CA1

Authors: *J. FOURNIER, A. B. SALEEM, K. D. HARRIS, M. CARANDINI;
UCL, London, United Kingdom

Abstract: CA1 place cells fire in specific locations in the environment, providing an animal with a continuous update of its position. Their firing reflects a combination of sensory and self-motion signals (Gothard et al., 1996; Jeffery et al, 1997; Redish et al., 2000). However, it is not clear how the combination of these two signals contributes to the representation of space by populations of CA1 neurons.

We investigated this question in a virtual environment, where visual and self-motion signals can be reliably manipulated. We trained mice running on a wheel to perform a navigation task in a virtual corridor. Mice learned to lick in specific positions of the corridor to obtain a water reward. We changed the gain of the wheel (i.e. by how much it displaced the virtual world) randomly by +/- 20% on a fraction of trials. While the mouse performed the task, we recorded from populations of CA1 neurons with silicon probes. These probes typically yielded recordings from populations of >50 well-isolated neurons.

Many putative pyramidal neurons (cells with complex spike bursts) exhibited typical properties of place cells, as described in freely-moving animals. They showed place fields specific to positions in the virtual corridor (Harvey et al, 2009; Chen et al, 2009) and place cell firing relative to theta phase revealed clear phase precession (O'Keefe & Recce, 1993). While place fields tiled the entire corridor, they appeared clustered, as if the corridor was encoded in segments instead of metric positions (Gupta et al., 2012).

To quantify the relative contribution of visual and self-motion signals on the encoding of position in CA1, we used a Bayesian decoder to predict the position of the animal from the neuronal ensemble in the different gain conditions. When we altered the gain of the wheel, the position decoded from the CA1 population was intermediate between the prediction based on

visual cues and the one based on self-motion cues. This effect was consistent with the animals' behavior: the distribution of licks was also intermediate between the predictions of the rewarded location based on vision and self-motion.
We conclude that the population of CA1 place cells encode both visual and self-motion signals in virtual reality, consistent with observations in real environments. We are currently exploring the nature of the interaction between these two streams of information on position encoding by CA1 neurons.

Disclosures: J. Fournier: None. **A.B. Saleem:** None. **K.D. Harris:** None. **M. Carandini:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NSF/ANR Collaborative Research in Computational Neuroscience Grant: Spaquence

Title: Prefrontal cortex reservoir network learns to create novel efficient navigation sequences by concatenating place-cell snippets replayed in hippocampus

Authors: *N. CAZIN[1,2], M. LLOFRIU ALONSO[3], P. SCLEIDOROVICH CHIODI[3], T. PELC[4], A. WEITZENFELD[3], J.-M. FELLOUS[4], P. F. DOMINEY[1,2];
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Abstract: Rats navigate efficiently between baited reward sites in an open field. From trial to trial, rats explore both inefficient path segments as well as segments that contribute to the final path. During inter-trial pauses, place fields that have been recently traversed reactivate in short sequences ("snippets") in the hippocampus. We hypothesize that this replay exposes prefrontal cortical circuits to subsequences of the final sequence that should be generated. We test the hypothesis that by using efficient segments that include a reward, the reservoir can learn to reconstruct the efficient final trajectory. We model PFC as a recurrent reservoir network of leaky integrator neurons, with fixed recurrent inhibitory and excitatory connections, and modifiable readout connections. Readout neurons feed-back into the reservoir, allowing for autonomous sequence reproduction and generation. Hippocampal place cells are distributed in a 16x16 grid over the area. Trajectories linking the 5 wells are used to generate place cell snippets, allowing us to perform learning experiments. We separately study snippet concatenation, selection and

recombination in a series of simulations. For snippets that have place fields with sufficient spatial overlap, the PFC reservoir is capable of concatenating them in order to generate the target sequence, even if it has never been seen in the training data. When snippets are generated from two sequences of different lengths, with more overlap between subsequences, the system selects and generates the shorter sequence. We then generated snippets from multiple sequences that were not the target sequence, but that contained components of the target sequence. Snippets associated to a reward had a higher probability of being replayed than others snippets. Thus, trajectory components being a part of a rewarded path were favored, and the PFC reservoir discovered the efficient sequence. The simulations were compared to rat data and we found good but not complete agreements. While being driven with the target sequence, the model produced predictions of the next place cell firing pattern that were spatially coherent with an efficient sequence but failed at generating an efficient sequence when driven only with the previous prediction at each simulation step. These results indicate that (1) recombination capabilities of the cortical reservoir contribute to target sequence identification, and that (2) it is likely that additional cues (e.g. visual perception of the arena) also contribute to the generation of the target sequence.

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Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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Sloan Foundation Research Fellowship

NARSAD Young Investigator Award (Brain and Behavior Foundation)

Title: Contralateral inactivation of the dorsal hippocampus and prefrontal cortex using dreadds impairs spatial learning

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Abstract: Coordinated activity between the hippocampus and the medial prefrontal cortex (mPFC) is critical for learning and memory-guided behavior. The hippocampus and prefrontal cortex support memory formation and retrieval, working memory, planning, and decision making. Several functional disconnection studies have previously reported spatial memory impairments using contralateral inactivation of the mPFC and the intermediate or ventral hippocampus (*Floresco et. al*, *1997; Wang et. al, 2006; Wang et. al, 2008*; *Churchwell et al. 2010*). Contralateral inactivation disrupts hippocampal-prefrontal interactions by functionally disconnecting ipsilateral projections between the regions. Theta oscillations and sharp-wave ripples (SWRs) have been have been implicated as the physiological mechanisms underlying hippocampal-prefrontal interactions, and we have shown that these network patterns mediate communication between these regions during learning of a W-track spatial alternation task (*Siapas et. al, 2005*; *Gordon*, *2011*; *Jadhav, et al., 2016*). This communication may be supported by minor projections between dorsal hippocampus and prefrontal areas, and due to the dense interconnectivity throughout the longitudinal axis of the hippocampus (*Cenquizca and Swanson, 2007; Witter and Amaral, 2004*). Here, we tested if contralateral inactivation of the mPFC and the dorsal hippocampus leads to impairment in learning of the W-track task. We used a chemogenetic approach to contralaterally inactivate the mPFC and the dorsal CA1 regions of the hippocampus in adult Long Evans rats during learning of a W-track spatial alternation task. Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) were transduced with the use of viral vectors to specifically target the prelimbic, infralimbic and anterior cingulate cortices of the mPFC in the right hemisphere of the rodent brain, and the dorsal CA1 layer of the hippocampus in the left hemisphere of the brain. Clozapine-*N*-oxide (CNO) injections were used to inactivate these regions during learning. We found that the animals with DREADDs inactivation were impaired in the outbound, spatial working memory component of the task as compared to control animals. Our results thus provide behavioral evidence that functional disconnection of mPFC and dorsal hippocampus via contralateral inactivation impairs the ability of animals to learn a spatial working memory dependent task.

Disclosures: **D.M. Maharjan:** None. **E.H. Glantz:** None. **Y. Dai:** None. **S.P. Jadhav:** None.

Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

Location: Halls B-H

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Support: Medical Research Service of the Department of Veterans Affairs

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NSF Temporal Dynamics of Learning Center Grant

Neuroplasticity of Aging Training Grant

Title: The role of the hippocampus and medial prefrontal cortex in path integration, spatial alternation, and nonspatial alternation

Authors: *M. SAPIURKA[1,2], L. R. SQUIRE[3,2,4,5], R. E. CLARK[3,2];
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Abstract: Working memory refers to the limited amount of information that can be held in mind with active maintenance, and is thought to be independent of the hippocampus and the adjacent structures of the medial temporal lobe (MTL). Working memory capacity is limited by the complexity of the information being maintained. While working memory can support the recall of 7 digits, its capacity for more complex stimuli is far more limited (i.e. 3-4 objects and 1 face). A recent study (Kim, Sapiurka et al, 2013) found that patients with damage to the MTL were intact at a spatial navigation task, path integration, presumably by using their intact working memory. In contrast, rodents with lesions of the hippocampus were impaired at path integration, even when trials took fewer than 3 seconds to complete, were less than 1 m in length, and involved no turns. One explanation for these results is that, while human working memory capacity is sufficient to support path integration performance, rodents may be unable to construct a working memory of a complex spatial environment. Accordingly, rats need to rely on long-term memory to successfully path integrate. To explore this possibility, we first tested rats with lesions of the medial prefrontal cortex (mPFC) on path integration and on spatial alternation, a test of spatial working memory. Lesions of mPFC have been associated with impaired working memory. If path integration requires long-term memory, there might be no effect of mPFC lesions on task performance. Indeed, despite impaired performance on spatial alternation, rats with mPFC lesions performed as well as controls at path integration. For comparison, we also tested rats with hippocampal lesions on spatial alternation. Hippocampal lesions robustly impaired performance on spatial alternation. To further test the effects of mPFC and hippocampal lesions on working memory, we developed a nonspatial working memory task, odor alternation, in which animals alternated choices between two differently scented objects. Rats with hippocampal lesions performed as well as controls on odor alternation, while rats with mPFC lesions performed no better than chance. We suggest that rats with hippocampal lesions are impaired on spatial memory tasks because of their limited working memory and impaired long-term memory not because of a broad inability to navigate or to process spatial information.

Disclosures: **M. Sapiurka:** None. **L.R. Squire:** None. **R.E. Clark:** None.

Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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Topic: H.01. Animal Cognition and Behavior

Support: UCRF

PCLB Foundation

Title: Inactivation of medial prefrontal cortex, dorsal, or ventral hippocampus during a temporal sequence task in a radial arm water maze

Authors: *S. LEE[1,2], J. R. PFLOMM[2], E. J. MARKUS[2];
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Abstract: The hippocampus and medial prefrontal cortex have been linked to memory formation, specifically working and episodic memories. These types of memories both have a temporal component. To further examine temporal processing we developed a temporal sequence order task in an 8-arm radial water maze. Rats experienced multiple maze sessions in the same room. Each session had a different fixed correct goal arm; however the room and maze were kept identical. Therefore to identify the correct goal for a given session one would need to remember the number of previous sessions already experienced that day.
We have previously shown that rats can learn up to seven locations in this task, and these seem to be learned as a sequence with more errors in the middle sessions than in the beginning and end sessions.
The current experiment used Muscimol to temporarily inactivate the dorsal/ventral hippocampus or medial prefrontal cortex in a within-animal repeated design. Animal testing concluded with infusions of fluorescent muscimol to visualize the spread of inactivation.
Male F-344 rats (N=9), approximately six months old, were trained in an eight-arm radial water maze with a removable escape platform. Rats were taught the first arm in the sequence, and additional arms were added when rats mastered the previous arm(s). After initial exposure to the maze and task, swim latencies stabilized to five seconds for a rat to select their first arm choice. Rats successfully selected the correct goal location in less than one-minute.
Prior to surgery, rats were trained on a three-arm sequence. Post surgery after their performance stabilized, the animals were tested under muscimol and control conditions. Initial results indicate that medial prefrontal cortex inactivation impaired performance for even the first session. Hippocampal inactivation had much less of an impact on performance. The complete finding and implications will be presented and discussed within the context of temporal processing.

Disclosures: **S. Lee:** None. **J.R. Pflomm:** None. **E.J. Markus:** None.

Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 554.16/LLL56

Topic: H.01. Animal Cognition and Behavior

Support: University of Connecticut PCLB

Title: Influence of the social environment on the behavior of rat pairs exploring a novel open field

Authors: *L. HORBAL, S. AHMED, S. LEE, E. J. MARKUS;
Dept. of Psychological Sciences, Behavioral Neurosci. Div., Univ. of Connecticut, Storrs, CT

Abstract: Exploration of a new environment is an essential component of animal behavior, providing potentially crucial information regarding sources of food, shelter, and a mates. The manner in which rats explore a novel environment has been examined previously using the open field task. Recent work by Weiss and colleagues indicates that rats may explore familiar arenas differently in the presence of another rat (Weiss et al., Animal Cognition, 2015 18(1), 39-51). They reported rats acting as either the “leader” or “follower”, where the leader primarily explores, while the follower is focused on the behavior of the leader. We sought to further examine differences in behavior between single rats and dyads during the exploration of an environment novel to the animals. Nine month-old male F344 rats (Harlan, IN), on a 12hr light/dark cycle with free access to food and water. Animal were paired for 10 days prior to and throughout testing. The open field exploration was assessed using a Plexiglas enclosure (70.5cm L X 70.5cm W) with a white plastic floor of the same area. During experimental trials, either one or two rats were placed in the southeast corner of the open field, and allowed to explore for fifteen minutes. The positions of the subjects within the open field were recorded using SMART-TW video multiple subjects tracking software (Panlab, Spain). The software allowed for tracking of multiple subjects simultaneously, each one physically marked with a different color. The head and back of each rat was tracked separately using two different colors.
Animals were placed in the novel environment either individually or in pairs. The pairs were either cage-mates or “strangers”. Exploration pattern on the first (novel) and second (familiar) days was compared, as was the interactions between the rat pairs.

Disclosures: **L. Horbal:** None. **S. Ahmed:** None. **S. Lee:** None. **E.J. Markus:** None.

Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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Topic: H.01. Animal Cognition and Behavior

Support: NEI R01EY022062

Title: Cortico-hippocampal neural activity during self-movement: navigating from heading to place

Authors: *W. K. PAGE, C. J. DUFFY;
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Abstract: Navigation relies on the real-time integration of self-movement cues to update the internal representation of the observer's location in the environment. Environmental vision provides a variety of self-movement cues, most prominently, the radial patterns of visual motion in optic flow that reflect heading direction and environmental layout. Neurons in medial superior temporal cortex (MST) are responsive to optic flow, combining those responses with visual signals derived from the relative movement of earth-fixed objects and vestibular signals about self-movement. In contrast, hippocampal neurons are selectively activated when the animal's is in a particular room location, with the net effect of creating a hippocampal map of extrapersonal space. We hypothesize that MST's self-movement responses are integrated to generate a hippocampal representation of extrapersonal spatial location. To test this hypothesis, we trained monkeys in an active steering to location task and recorded neural activity in MST and hippocampus. The monkey's chair was mounted on a room-sized, motorized, 2-axis translational sled. The monkey had free-viewing of the room and of a small video display which simulated a top-down view of the room. The video display could indicate the monkey's current room position and the current goal location. The monkey used a 2D joystick to steer sled movement, attain the target goal location, and earn liquid reward. Neural signals were recorded during and between self-movement excursions. MST cortical and hippocampal single neurons, and intracranial evoked potentials, were recorded during stroboscopic and continual room illumination. We find that the stroboscopic illumination yields ERPs reflecting self-movement. Continual illumination shows MST neuronal responses to self-movement whereas hippocampal neurons show room location dependent activity. These findings suggest that MSTd neurons are well-suited to providing self-movement signals supporting hippocampal place activity.

Disclosures: W.K. Page: None. **C.J. Duffy:** None.

Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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Title: Strategy-specific patterns of Arc expression in the hippocampus and retrosplenial cortex during T-maze learning

Authors: *R. CZAJKOWSKI[1], B. ZGLINICKI[2], E. REJMAK[1], W. KONOPKA[2];
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Abstract: Spatial memory depends on several interconnected brain regions with spatially tuned neurons. Hippocampus harbors place cells that form the spatial map of the environment. Retrosplenial cortex (RSC) contains neurons that process information about the direction (head direction cells). The precise function of RSC in the spatial memory network is still a matter of debate. T-maze is one of the most commonly used behavioral tasks that allow testing rodent spatial memory and navigation. In the first phase of each training trial the animal is forced to make a turn into selected arm and collect a reward. In the second phase, both arms are open and only the previously unvisited arm is rewarded. We modified the T Maze design in order to increase the dependency on allothetic, extramaze cues. Walls were made of transparent plexiglass and a large projection screen was placed in front of the maze. Animals were trained to follow two sequences (right-left or left-right) based on two images projected onto the screen (context A or B, respectively). Almost all animals (13 out of 14) learned to perform both versions of the task within 16 sessions (8 trials per session). During the test phase, the context was replaced between forced and choice phase. In half of the animals (7 out of 13) this resulted in revisiting the arm, consistently with the changed spatial cues. 48 hours later the animals were subjected to one regular trial in each of the contexts with 18 minutes interval between trials. 2 minutes after second trial animals were sacrificed and brains were processed for in situ hybridization (catFISH) with a probe for immediate early gene Arc. The number of Arc-positive nuclei (activated only in trial 2) and double positive cells (nucleus and cytoplasm, activated in both trials) was counted for RSC and hippocampal regions: dentate gyrus, CA1 and CA3. The overall number of Arc positive nuclei was significantly higher in the animals that did not follow spatial cues in both agranular RSC (6.82% vs 3.25%, $p<0.05$) and in granular RSC (4.43% vs 1.86%, $p<0.05$). There were no differences observed in dentate gyrus, CA1 or CA3. No differences in the level of overlap between cytoplasm and nuclei labeling was observed in any of the regions, except for CA3 where higher overlap between context representations was observed

for animals expressing the non-spatial strategy ($p<0.05$). Since Arc is commonly associated with learning – dependent plasticity, these results suggest that the non-spatial animals were acquiring the spatial strategy (high level of Arc in RSC) and the spatial representations for both contexts were still indistinguishable in the non-spatial animals (high overlap of Ctx A and B).

Disclosures: R. Czajkowski: None. **B. Zglinicki:** None. **E. Rejmak:** None. **W. Konopka:** None.

Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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Title: Cholecystokinin from the entorhinal cortex switches high-frequency-induced long-term potentiation in the hippocampus

Authors: *J. SU[1], W. YE[2], X. CHEN[1], H. FENG[1], P. JANDRICHOVSKY[1], J. HE[1]; [1]City Univ. of Hong Kong, Hong Kong SAR, China; [2]Guangzhou Inst. of Biomedicine and Health, Chinese Acad. of Sci., Guangzhou, China

Abstract: Our earlier studies showed that activation of cholecystokinin (CCK) neurons that originated from the entorhinal cortex induce long-term potentiation and neuroplasticity in the auditory cortex. Lesions in the entorhinal cortex or the hippocampus will lead to memory deficits which have received intensive studies, including temporal, spatial, episodic memory, as well as associative fear conditioning. After AAV-DIO-ChR2-eYFP injected into the entorhinal cortex of the CCK-Cre mouse, we discovered that entorhinal CCK neurons project to hippocampus

regions. We hypothesized that CCK is involved in the high-frequency stimulation induced long-term potentiation (LTP). High-frequency electrical stimulation (in Theta Burst format) in Schaffer collateral induced CA3-CA1 LTP on the wild-type mouse and CCK B-receptor knockout (CCK-BR KO) mouse, but not on the CCK peptide knockout mouse (CCK-KO). It was interesting to note that the hippocampus has many CCK A-receptors (ref. Allen Brain Atlas), which might be the reason of why CCK-BR-KO still has High-frequency stimulation induced LTP. The CCK-KO mouse exhibited severe fear memory and spatial memory deficit. On the CCK-Cre mouse with injection of AAV-DIO-ChR2-eYFP in the entorhinal cortex, we induced CA3-CA1 LTP with Low-frequency electrical stimulation on Schaffer collateral after High-frequency laser stimulation of the projection terminals originated from the entorhinal cortex. We explain that High-frequency stimulation of the CCK terminals induced CCK release in hippocampus, and LF stimulation could induce LTP in the presence of CCK.

Disclosures: **J. Su:** None. **W. Ye:** None. **X. Chen:** None. **H. Feng:** None. **P. Jandrichovsky:** None. **J. He:** None.

Poster

554. Hippocampal-Cortical Interactions in Spatial Cognition

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Topic: H.01. Animal Cognition and Behavior

Support: CAPES (Brazil) PhD Stipend 9694-13-7

Title: *In vivo* identification of brain structures implicated in spatial learning and memory, and its modulation by emotional drive in mice

Authors: *S. ALMEIDA-CORREA[1], B. T. BEDENK[2], A. GENEWSKY[1], S. A. BURA[1], J. DINE[1], M. CZISCH[1], C. T. WOTJAK[1];
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Abstract: Cognition might be impaired during negative affect. Specially, hippocampus-dependent memories, such as spatial learning, can be dramatically disrupted by emotional drive. Most information concerning this matter is based on *ex vivo* analyses. Given the impact of these impairments at the individual's life, it is important to understand the interplay between emotion and cognition *in vivo*. With that in mind we aim to investigate the role of emotionality on cognitive performance in the water cross maze (WCM), to identify the brain structures involved in these processes and to demonstrate their mutual interaction. Specifically, (i) we performed *in vivo* identification of brain structures involved in hippocampus-dependent (place based) versus

hippocampus-independent (response based) spatial learning, through MEMRI analyses after WCM training; (ii) we will compare these *in vivo* (MEMRI) data and *ex vivo* (IEG) analyses of neural activity for learning, to complement the dissection of neuronal populations implicated in the different spatial learning strategies; (iii) validate the causal involvement of (a) the brain structures identified in spatial learning, with particular emphasis on consequences of the modulation of activity (though DREADDs) in brain structures implicated in stress and anxiety (i.e. amygdala) and memory (i.e. hippocampus); (b) of neuronal circuits in emotional control of spatial learning (pathway analysis), through optogenetics, in specific targets, previously identified by MEMRI. We believe that these investigations will contribute to the understanding of the impact of emotionality on cognition, therefore elucidating some basic aspects of psychiatry disorders in which these detrimental effects of persistent arousal are present.

Disclosures: S. Almeida-correa: None. **B.T. Bedenk:** None. **A. Genewsky:** None. **S.A. Bura:** None. **J. Dine:** None. **M. Czisch:** None. **C.T. Wotjak:** None.

Poster

555. Perception and Imagery

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 555.01/LLL61

Topic: H.02. Human Cognition and Behavior

Title: Modulation of primary motor cortex inhibition during motor imagery

Authors: *F. LEBON[1], C. STINEAR[2], C. PAPAXANTHIS[1];
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Abstract: Motor imagery is a specific cognitive state during which the corticomotor system is specifically involved. However, one paradox remains unsolved: how the facilitation of the corticomotor pathway during motor imagery does not produce descending motor output? One hypothesis is that inhibition is recruited during motor imagery to prevent descending motor output (Jeannerod, 1994). An alternative explanation is that during motor imagery the level of activation is insufficient to produce a descending motor command (Guillot et al., 2012).
We used transcranial magnetic stimulation (TMS) to probe the modulation of inhibitory circuits within the primary motor cortex at rest and during imagined movement of the index finger of the right hand (electromyographic electrodes positioned on the First Dorsal Interosseus muscle). Single-pulse TMS was delivered over the left hemisphere to assess corticomotor excitability. Paired-pulse TMS with 2 ms and 100 ms inter-stimulus intervals was used to assess short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI), respectively. Finally, we stimulated the median nerve 23 ms prior to single and paired-pulse

TMS to test the effect of low-threshold peripheral nerve stimulation on cortical processes by measuring short-interval afferent inhibition (SAI).
As expected, motor imagery increased corticomotor excitability and reduced SICI (Lebon et al., 2012; Stinear et al., 2004). We also found that motor imagery decreased LICI, demonstrating that this cognitive process acts on both fast and slow inhibitory neural mechanisms within the primary motor cortex. Then, we tested the same mechanisms with stimulation of the median nerve to elicit afferent inputs. We replicated previous results showing the presence of SAI at rest: the conditioning peripheral nerve stimulation decreased single-pulse MEPs (motor-evoked potentials) and reduced SICI and LICI (Udupa et al., 2009 and 2014). Interestingly, motor imagery reduced the inhibitory effects of SAI in comparison to rest: greater single-pulse MEPs and greater SICI and LICI. This would mean that the activation of sensory brain areas observed during motor imagery (Hetu et al., 2012) may block the effect of afferent inputs onto motor cortex.
While the increase of corticomotor excitability during motor imagery may originate from the reduction of intracortical inhibitory mechanisms (SICI and LICI), the concomitant activation of associated motor brain areas, such as the sensory cortex, may reduce this activity to prevent descending motor output, and therefore actual execution.

Disclosures: **F. Lebon:** None. **C. Stinear:** None. **C. Papaxanthis:** None.

Poster

555. Perception and Imagery

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Max Planck Society Grant

Title: Body schema boundaries are formed by sensorimotor body-environment distinction.

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Abstract: The basic self-perception of being a body that is delimited and in this sense distinct from the environment should be defined by the boundaries of the body schema. The body schema was and is extensively investigated in humans, e.g., in studies on neurological disorders,

as well as in animals, e.g., in studies on somatosensory receptive fields. Yet, it was so far not investigated how the boundaries of the body schema are formed. We have investigated if these subjective body boundaries result from the distinction of body and environment sensations regarding their predictability by the motor representation of active bodily self-motion. In a first study, we enabled healthy humans to control with their physical body a life-sized virtual body that was mirroring their movements in extrapersonal space. In a second study, we asked human subjects to control either such a mirror-avatar or one that was additionally unpredictably shaking. We found that the remote motor control of the mirror-avatar caused the body schema boundaries to decline while inhibiting the distinction of auto-kinesthetic sensations that are, and visual motion sensations that are not, directly predicted by the motor representation of active bodily self-motion. This sensorimotor body-environment distinction was instead intensified by the remote motor control of the shaking-avatar, which fortified the body schema boundaries. Our findings suggest that the distinction of sensations that are, and those that are not, directly predicted by motor representation based on their motor predictability is forming the human body schema boundaries. This sensorimotor body-environment distinction corresponds to the previously suggested self-world distinction that is regarded as fundamental for motor control to arise from sensorimotor integration and for body self-perception in general. Such a sensorimotor distinction and the body schema boundaries that it forms may accordingly affect the sense of agency and various other body self-perception components such as the sense of bodily self-identification and self-location.

Disclosures: **M. Dobricki:** None. **B.J. Mohler:** None.

Poster

555. Perception and Imagery

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Topic: H.02. Human Cognition and Behavior

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Title: Virtual embodiment enhances brain motor resonance mechanisms

Authors: ***M. SLATER**[1,2], M. GONZALEZ-FRANCO[3], J. ARROYO-PALACIOS[3], R. KATZ[4], A. RODRIGUEZ-FORNELLS[1];
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Abstract: Introduction: Using immersive virtual reality (IVR) it is possible to visually substitute a person's body by a life-sized virtual body (VB), seen from first person perspective (1PP), and which moves synchronously and in conformity with a person's real movements, as seen through a head-tracked head-mounted display (HMD), and real-time motion capture. Such a setup has been used to investigate body ownership and illusory agency, where participants attribute actions of the VB to themselves. We carried out an experiment to explore aspects of brain activity (surface EEG signals) when a virtual arm moves. The objective was to understand the relationship between real, and virtual arm movements. Given that previous research has shown that the mirror neuron mechanisms are more active from 1PP our hypothesis was that VB ownership might increase those effects.
Method: The experiment had 4 conditions: Motor execution in VR (ME1PP): participants performed the motor action with each arm in turn, while the virtual arm moved synchronously, with the VB seen from 1PP. Motor observation in 1PP (MO1PP): participants saw their VB from 1PP, and observed the motor action while not moving themselves. Motor observation in 3PP (MO3PP): participants saw the VB from third person perspective (3PP) and observed the motor action while not moving themselves. Real arm observation (RME): participants performed the motor execution looking at their real body without VR. In each case the left and right arm moved 80 times. There was a 64-multichannel EEG recording (amplifier g.Tec Medical Engineering). There were 18 right-handed healthy male participants who completed all 4 conditions.
Results: The lateralized readiness potential (LRP) was studied as it is a sensitive marker of motor execution and it has been previously associated with the activation of the mirror neuron system. A significant increase in the amplitude of the LRP was found for both motor execution conditions (RME and ME1PP). Importantly, no differences were observed between these conditions indicating that the LRP was equivalent in the real virtual environment during action execution (notice that in both cases participants were moving their arms). Although the amplitude of the LRP during the observation conditions is reduced compared to the real execution conditions, the MO1PP has an earlier activation (onset latency) than the MO3PP condition, being more aligned with the temporal dynamics observed in the case of the real motor execution.
Conclusion: This suggests that strong body ownership while observing the virtual arm moving may contribute towards motor resonance mechanisms.

Disclosures: M. Slater: None. **M. Gonzalez-Franco:** None. **J. Arroyo-Palacios:** None. **R. Katz:** None. **A. Rodriguez-Fornells:** None.

Poster

555. Perception and Imagery

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 555.04/LLL64

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI 26750242

JSPS KAKENHI 26242065

Title: The right temporoparietal junction encodes efforts of others during action observation

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Abstract: Smooth social interactions require a deep understanding of others' intentions and feelings. In the present study, to investigate brain regions that respond to inference of others' effort level, we recorded brain activity during action observation of different effort levels using functional magnetic resonance imaging (fMRI). We used a dumbbell curl movement to depict a movement requiring effort. To dissociate the factors of effort level of the actor and weight of the dumbbell, we used four combinations of dumbbell weight and actor physique: a thin actor or a built actor lifting a heavy or light dumbbell. During observation of dumbbell curls, the bilateral front-parietal action observation network (AON) was activated. This included the premotor cortices, parietal cortices, visual areas 5/superior temporal cortices (STS), amygdalae, hippocampi, right dorsolateral and ventrolateral frontal cortices. When we evaluated brain regions associated with the actor's effort level, activity in the right temporoparietal junction (TPJ) and STS was observed. However, activity in the front-parietal AON was independent of the actor's effort during action observation. This finding suggests that the right TPJ and STS play an important role in the inference of others' effort levels during the observation of others' movements.

Disclosures: **N. Mizuguchi:** None. **H. Nakata:** None. **K. Kanosue:** None.

Poster

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Program#/Poster#: 555.05/LLL65

Topic: H.02. Human Cognition and Behavior

Title: Spectral fingerprint of heartbeat-related brain responses in the human somatosensory cortex revealed by ECoG

Authors: *M. J. KERN, F. MANZOURI, A. SCHULZE-BONHAGE, T. BALL;
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Abstract: Introduction: The cortical processing of cardiac action-related activity is an important viscero-afferent process in the human brain. Consequently, the investigation of heartbeat-related brain responses, especially in the context of heartbeat perception, has become a prominent topic in neuroscience research. Just recently, for the first time a focally localized cortical heartbeat-evoked potential (HEP) within the human primary sensory cortex and its precise time course were described in ECoG (Kern et al., 2013). Although the HEP is well described in the time domain, there is a lack of data on its fingerprint in the frequency domain. Hence, the aim of this study was to characterize heartbeat-related brain responses by ECoG, especially in the frequency domain.
Methods: For the analysis of heart cycle-related ECoG changes, we used heartbeats from recordings of epilepsy patients during natural behavior. A large number of heartbeats (up to ~7000 per patient) were used, without additional burden on the patients, allowing a heart-rate-dependent analysis. For the time frequency analysis, we used a multi tapered time resolved fast Fourier transformation.
Results: First, we replicated our results from 2013 and detected HEPs in the primary sensory cortex in several additional patients. Second, we observed a negative correlation of heart rate and HEP amplitude, while the time course of the HEP remained unaffected. Third, our new findings revealed a prominent heartbeat-related changes in the alpha (8-13Hz), beta (15-35Hz), and gamma (>50Hz) frequency bands within the primary sensory cortex. The spatiotemporal pattern of the heartbeat-related spectral magnitude changes was in good accordance with the pattern of HEPs and its magnitudes were also negatively correlated to the heart rate.
Conclusions: Our findings indicate the existence of heartbeat-related neuronal signals in the alpha, beta, and gamma frequency domain, and with a topography further supporting the hypothesis of cardiac-related information processing via a somatosensory pathway. These signals existed although the patients were not specifically instructed to either try to perceive their heartbeats or to ignore it. Thus, the processing of cardiac-related information seems to unconsciously proceed in natural, non-experimental conditions and manifests itself in distinct cortical responses both in the time and frequency domains.

References:
Kern, M., Aertsen, A., Schulze-Bonhage, A., Ball, T., 2013. Heart cycle-related effects on event-related potentials, spectral power changes, and connectivity patterns in the human ECoG. NeuroImage 81, 178-190.

Disclosures: **M.J. Kern:** None. **F. Manzouri:** None. **A. Schulze-Bonhage:** None. **T. Ball:** None.

Poster

555. Perception and Imagery

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Program#/Poster#: 555.06/LLL66

Topic: H.02. Human Cognition and Behavior

Title: Mind-wandering in sensory cortices

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Abstract: Mind-wandering occupies a significant amount of our waking time and has been shown to be detrimental to task performance. However, the nature of mind-wandering and its relationship to sensory cortices remain elusive. Here we utilized fMRI and online thought sampling to examine the neural correlates of mind-wandering. Specifically, the thought sampling procedure probed participants to report mind-wandering events and their corresponding modalities (e.g. visual/auditory) while they underwent a fixation task during scanning. The 9-second period prior to each probe was extracted and labeled as mind-wandering or non-mind-wandering events, depending on how focused they were on the fixation task. Whole brain analysis comparing mind-wandering versus non-mind-wandering events showed stronger mean BOLD (blood-oxygen-level dependent) activation in regions involved in the default mode network, including posterior and anterior cingulate cortices. Critically, when we compared events in which participants reported having visual-only or auditory-only contents, visual cortex (i.e. Cuneus) and auditory cortex (i.e. Transverse temporal gyrus) were more activated, respectively. Furthermore, early visual cortex (i.e. v1) exhibited distinct pattern activity and stronger mean BOLD activation when comparing events with visual contents to non-mind-wandering events. Our results suggest a strong link between mind-wandering and sensory cortices as mind-wandering of specific sensory contents recruits its corresponding sensory cortices.

Disclosures: **S. Hung:** None. **P. Hsieh:** None.

Poster

555. Perception and Imagery

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Topic: H.02. Human Cognition and Behavior

Support: Studienstiftung des Deutschen Volkes

Investissements d'avenir ANR-10-IAIHU-06

Title: Hypnotic states induce high-frequency power changes (70-140 Hz) in the default mode network

Authors: *J. CORLIER[1,2], C. BERNARD[3], C. FLAMAND-ROZE[3], V. BOUILLERET[3], V. NAVARRO[1,2,4], I. CELESTIN-LHOPITEAU[3,5], M. LE VAN QUYEN[1,2];
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Abstract: Hypnotic suggestion is being increasingly used clinically, especially because of its efficacy in stress, anxiety and pain management [1]. Recent works suggest that hypnosis produces alterations in the default mode network (DMN), probably related to the changes in self-referential processing [2]. In this study, our aim was to investigate the fine-scale temporal dynamics of local-field potentials within the DMN during hypnosis. Taking advantage of invasive intracerebral recordings of epileptic patients undergoing a diagnostic evaluation in a presurgical context, we recorded in total from 10 patients and 690 fronto-temporal cortical sites, while the patients were undergoing a hypnotic induction by an experienced hypnotherapist. Focusing on the high-gamma power (HGP, 70-140 Hz that was previously shown to reflect changes in DMN-activity, [3]) and following the subdivision of the DMN into distinct functional-anatomic components proposed by [4], our results show: 1) a reduction of HGP in medial frontal components of the DMN, possibly reflecting a reduction in self-referential cognitive activity, 2) increases in HGP in the medial temporal DMN-component, presumably reflecting increased retrieval of autobiographic memories or imaginary scenarios; 3) increase of HGP at fronto-parietal locations of the attentional network indicating a progressive absorption during the hypnotic induction and also 4) HGP changes in the amygdala suggesting changes in emotional processing. In sum, our findings indicate that hypnosis has a modulatory effect on regions processing explicit cognitive control, memory recall, emotional and attentional processing. Selective targeting of these changes may be used to improve outcome in psychotherapy, pain control or in learning and memory function [5].References: [1] Patterson &

Jensen (2003); [2] Demertzi et al. (2011); [3] Ossandon et al. (2011); [4] Andrews-Hanna et al. (2010); [5] Nemeth et al. (2013)

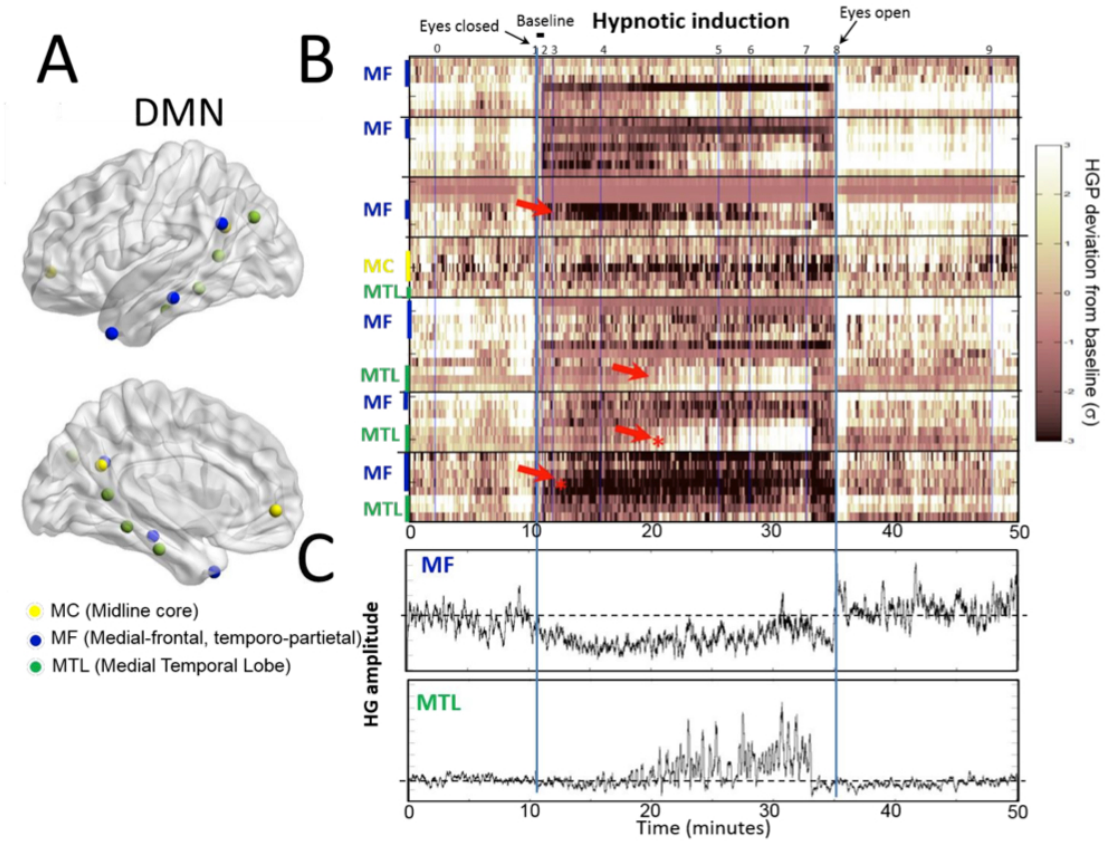

Figure 1: Activation and Deactivation in the DMN. A. Anatomical position of three subsystems of the DMN as described in [8], referring to the midline core (yellow), dorsal medial frontal/temporo-parietal (blue) and the medial temporal (green) clusters. B. Representation of changes in HGP across the hypnotic induction. Note the increase in the MTL and the simultaneous decrease in the MF subsystems (red arrows). C. Two examples of electrodes in the MF and the MTL displaying HGP decrease and increase, respectively. Electrodes correspond to positions marked with * in B.

Disclosures: J. Corlier: None. **C. Bernard:** None. **C. Flamand-Roze:** None. **V. Bouilleret:** None. **V. Navarro:** None. **I. Celestin-Lhopiteau:** None. **M. Le Van Quyen:** None.

Poster

555. Perception and Imagery

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Topic: H.02. Human Cognition and Behavior

Title: Individual differences in unconscious processing capacity

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Abstract: Experimenters have used a variety of masking techniques to demonstrate the presence or absence of unconscious processing. There is a growing body of evidence that the degree to which a stimulus is processed without conscious awareness depends on the choice of masking technique. Could there also be individual differences in the degree to which a stimulus is processed unconsciously? We replicate three unconscious semantic priming studies that used different masking techniques - Continuous Flash Suppression (CFS), object substitution masking, and the attentional blink - administered in counterbalanced order to the same group of subjects. We found a main effect of masking technique; for example, there was evidence for unconscious priming under object substitution, but not under CFS. The typical interpretation of this result would be that unconscious semantic priming does not occur under CFS. However, this null effect of the prime is the mean of a distribution; i.e., data are averaged across subject at some point in the analysis. We instead looked at the within-subject correlation of effect size across different choices of masking technique choice. The amount of unconscious priming a subject experienced in each suppression technique was significantly correlated. In other words, it is possible that the distribution of unconscious priming effects in an experiment yielding a null effect of unconscious priming is not entirely a result of random noise, but instead a combination of random noise and individual differences in amount of unconscious processing or susceptibility to various types of masking.

Disclosures: **N. Root:** None. **A. Rasheed:** None. **V. Ramachandran:** None.

Poster

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Prin 2010 (Italian Governement)

French Speaking Community Converted Research Action

Wallonie-Bruxelles International

Title: The fade in consciousness in anesthesia is associated with loss of deterministic responses to transcranial magnetic stimulation

Authors: *M. DARRACQ[1], M. BOLY[2], O. GOSSERIES[2], M.-A. BRUNO[3], V. BONHOMME[3], J.-F. BRICHANT[4], M. ROSANOVA[5], S. LAUREYS[3], G. TONONI[2], M. MASSIMINI[5], R. D. SANDERS[2];
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Abstract: Phase Locking Factor (PLF, also known as inter-trial coherence) analysis of Transcranial Magnetic Stimulation electroencephalography (TMS-EEG) provides an index of deterministic phase-locked cortical activity. We hypothesized that relative to wake, average PLF calculated across the scalp would fade under anesthesia with ketamine and propofol. However, ketamine and propofol have distinct anesthetic profiles. While both induce unresponsiveness, in this setting propofol is associated with unconsciousness while ketamine is associated with dreaming (disconnected consciousness). Hence we further hypothesized that if consciousness is a graded phenomenon, the fade in PLF would be greater for propofol than ketamine. We calculated the change in PLF following TMS, directed at the superior parietal gyrus, compared to baseline (assuming a Rayleigh distribution of baseline values between -400 to -50ms before TMS pulse [$p<0.05$]) over 60 EEG channels. We averaged the function of PLF over time for all channels per subject (propofol n = 8; ketamine n = 6) per condition and computed the area under the curve (PLF-AUC, integral). Group level contrasts were conducted using paired t-tests for wake versus propofol, and wake versus ketamine. Data are presented as mean ± standard deviation. The paired t-test contrasts show that propofol (wake 101 ± 50, propofol 11 ± 8, $p=0.0007$) and ketamine (wake 72 ± 39, ketamine 39 ± 15, $p = 0.0337$) decreased PLF-AUC relative to wake. Next we contrasted the drug effects through analysis of the proportional change in PLF-AUC induced by the drug from wakefulness (to eliminate heterogeneity in wakeful values) using an unpaired T-test. While both ketamine and propofol decreased PLF-AUC, the propofol effect was more profound (90±7% vs. 49±27%, $p = 0.0127$). Anesthetics decrease average scalp PLF-AUC in a graded manner consistent with their graded effect of consciousness. Such a metric could have clinical utility in detection of anesthesia awareness. Furthermore the graded changes observed are consistent with the idea that consciousness fades, rather than shatters, under anesthesia. Preliminary tests suggest that PLF-AUC under ketamine and propofol is an index robust enough to measure changes using a reduced group of electrodes, implying the possibility of studying the spatial heterogeneity of the fade in PLF across these states.

Disclosures: **M. Darracq:** None. **M. Boly:** None. **O. Gosseries:** None. **M. Bruno:** None. **V. Bonhomme:** None. **J. Brichant:** None. **M. Rosanova:** None. **S. Laureys:** None. **G. Tononi:** None. **M. Massimini:** None. **R.D. Sanders:** None.

Poster

555. Perception and Imagery

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Topic: H.02. Human Cognition and Behavior

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French Speaking Community Concerted Research Action

Wallonie-Bruxelles International

Title: Loss of cross frequency coupling and hierarchical cortical connectivity in propofol-induced unresponsiveness.

Authors: *R. D. SANDERS[1], M. DARRACQ[1], O. GOSSERIES[2], M. BRUNO[2], V. BONHOMME[2], J. BRICHANT[2], M. ROSANOVA[3], R. MORAN[4], M. I. BANKS[1], G. TONONI[1], M. MASSIMINI[3], S. LAUREYS[2], M. BOLY[1];
[1]Univ. of Wisconsin, Madison, Madison, WI; [2]Coma Sci. Group, Liege, Belgium; [3]Univ. of Milan, Milan, Italy; [4]Virginia Tech. Carillion, Richmond, VA

Abstract: We hypothesized that the fade in consciousness that occurs under anesthesia involves impaired cross-frequency coupling and connectivity between hierarchical levels of cortex. This was tested using dynamic causal modelling (DCM) of occipital Transcranial Magnetic Stimulation electroencephalogram (TMS-EEG) data to model direct activation of a cortical network involved in perception and imagery and the subsequent integration of information between cortical areas. Eight subjects underwent occipital TMS-EEG recording during wakefulness followed by propofol titrated until loss of responsiveness to verbal command. TMS-EEG data were finite impulse response filtered at 0.5-40Hz, epoched, referenced to an averaged electrode, followed by sensor space, time frequency analysis. Merged data then underwent beamformer source reconstruction followed by DCM of the induced response from 1 to 400ms. Bayesian model selection (BMS) was used to optimize models for the analysis. DCM of the induced response employed an equivalent current dipole electromagnetic model. BMS supported full modulation of all connections between lower order inferior occipital gyrus (IOG) and higher order parietal (superior parietal lobule, SPL) and frontal sources (dorsolateral prefrontal cortex) and non-linear and linear cross frequency coupling. Statistical thresholds were set at Family Wise Error rate $p<0.05$. Sensor- and source-based time frequency analyses showed that propofol reduced evoked power following TMS at different frequencies in different regions. DCM, with full factorial analysis and F contrast across all connections, indicated that propofol-induced

unresponsiveness was associated with altered gamma and alpha-gamma cross frequency coupling. The change in gamma coupling involved an increase in ascending connectivity from IOG to SPL but decreased coupling across all descending connections. In contrast, the decreased alpha-gamma coupling involved ascending connections. Subsequent T contrasts for decreases in ascending and descending connectivity confirmed that descending connectivity was impaired in the gamma range while alpha-gamma ascending connectivity was impaired. Propofol-induced unresponsiveness was associated with spatial variation in evoked power, altered cross frequency coupling and disturbed bidirectional connectivity between hierarchical cortical areas. These data support theoretical advances suggesting consciousness fades during anesthesia due to altered integration of information between hierarchical levels of cortex.

Disclosures: R.D. Sanders: None. **M. Darracq:** None. **O. Gosseries:** None. **M. Bruno:** None. **V. Bonhomme:** None. **J. Brichant:** None. **M. Rosanova:** None. **R. Moran:** None. **M.I. Banks:** None. **G. Tononi:** None. **M. Massimini:** None. **S. Laureys:** None. **M. Boly:** None.

Poster

555. Perception and Imagery

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Program#/Poster#: 555.11/MMM1

Topic: H.02. Human Cognition and Behavior

Title: Default mode network activity analysis during self-contemplating image formation

Authors: *D. GUPTA[1], Q. MENG[1], E. HONG[2], F.-S. CHOA[1];
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[2]Univ. of Maryland Sch. of Med. and Maryland Psychiatric Res. Ctr., Baltimore, MD

Abstract: The wakeful resting condition is characterized by a default mode of brain function involving high levels of activity within a functionally connected network of brain regions. Studies indicate that this Default Mode Network (DMN) is the brain's baseline of information processing. Its activation represents how our brains consolidate experience and prepare to react to the outside environment, separate from the activity of conscious thought. Hence, DMN helps us make sense of our surrounding environment. Other work implicates that the associated cortical regions for contemplation and mind wandering, is essential for our consciousness and they bear thoughts not directly related to immediate external sensory inputs. In this work, we study and analyze the DMN using Electroencephalography (EEG), which provides higher temporal resolution as compared with other techniques such as PET and fMRI. This allows us to study the dynamics of different bands of brain-waves in DMN operations at the millisecond scale. In our study, subjects were asked to sit down quietly on a chair with eyes closed and think

about some parts of their own body, such as left and right hands, left and right ears, lips and nose etc. The moment when the image was formed in the subject's mind, he or she will mark the time. Next, the recorded brain electrical activity maps, 300ms prior to the time marked instant, were analyzed for each of the 4 wave bands namely, Delta, Theta, Alpha and Beta waves. We found that for most EEG datasets during this 300ms, Delta wave's activity lied mostly at the frontal lobe or the visual cortex and its movement across these brain regions were slow. On the other hand, theta wave's activity rotated along the edge of cortex in a clockwise and counterclockwise fashion. Furthermore, Beta wave acted as inquiry types of oscillations between any two regions of the cortex. Lastly, Alpha wave's activity looked like a mix of the Theta and Beta wave's activities along similarity with that of the Theta wave's activity. From these observations, we conclude that Beta and high Alpha are playing a role in information inquiry within the brain. Whereas, Theta and Alpha waves are more likely to play the role of developing and binding of imagination in the DMN operations.

Disclosures: **D. Gupta:** None. **Q. Meng:** None. **E. Hong:** None. **F. Choa:** None.

Poster

555. Perception and Imagery

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Program#/Poster#: 555.12/MMM2

Topic: H.02. Human Cognition and Behavior

Title: Graph theoretical analysis of functional connectivity network during breath-counting mindfulness meditation

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Abstract: Objective: Mindfulness meditation is a way of paying attention to one's experience in an accepting and nonjudgmental way. Practitioners try to observe their current experiences such as emotion, thoughts and sensations inside and around them. It is widely used as means of relaxation and stress reduction. The meditation requires our nonjudgmental observing, but its concept and instructions are often difficult for beginners. In this research, we investigated whether meditation beginners could derive any positive effect even by a simple way of focusing their breathing and counting them.
Methods: Three healthy male adults (average age: 22.33 ± 0.58 years, right-handed) with no experience of meditation were asked to mediate for 5 min in the functional magnetic resonance imaging (fMRI) scanner, by breath counting meditation. Participants performed the meditation

task after 5-min resting period, and closed their eyes consistently in the scanner. ROI-to-ROI functional connectivity was calculated using the functional connectivity toolbox (CONN), based on automatic anatomical labeling (AAL) atlas. Functional connectivity networks during resting and meditation states were analyzed based on graph theoretical measures of network centrality. Results: Analysis of an eigenvector centrality measure revealed that the centrality of five brain regions, superior occipital gyrus, left putamen, left pallidum, right superior temporal gyrus and middle temporal gyrus were differed between resting and meditation states. Superior occipital gyrus and superior temporal gyrus are associated with visual and auditory processing respectively, but their eigenvector centralities were decreased in meditation state compared with resting state. This might suggest that the participants could focus on their breathing, keeping the mind from wandering. Since putamen and palladium are part of basal ganglia and associated with voluntary motor control, the change of centrality of these regions suggested that they affected regulation of the rhythm of breathing. Middle temporal gyrus is related to mind-wandering, so that the meditation might reduce its centrality.
Conclusion: Graph theoretical analysis extracted the change of functional connectivity network from the resting state to the meditation state. Five brain regions, superior occipital gyrus, left putamen, left pallidum, right superior temporal gyrus and middle temporal gyrus were potentially crucial to distinguish between the resting and the meditation states. This result suggested the possibility that even a novice meditator could derive the benefits of meditation by a simple practice of counting the breath.

Disclosures: S. Hiwa: None. **M. Iizuka:** None. **T. Hiroyasu:** None.

Poster

555. Perception and Imagery

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Program#/Poster#: 555.13/MMM3

Topic: H.02. Human Cognition and Behavior

Support: NSERC Grant

Title: Functional connectivity in the salience network associated with trait mindfulness

Authors: *E. M. BILEVICIUS[1], S. D. SMITH[3], J. KORNELSEN[2,4];
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Abstract: Resting state networks are comprised of brain structures with activity that is highly correlated over a period of time. Researchers have identified a number of resting state networks in the brain and the salience network (SN) is one such network. It is important for the integration of sensory information and has keys nodes in the insula and anterior cingulate cortex (ACC). The SN's role in sensory integration makes its activity relevant to a number of phenomena including mindfulness. Neuroimaging investigations have looked at the structural and task-based functional changes that accompany mindfulness, which is defined as purposeful attention to moment-to-moment experiences with an accepting and nonjudgmental stance. However, relatively few studies have observed the neural substrates associated with trait mindfulness, an individual's intrinsic or fundamental level of mindfulness. In the current experiment, we examined whether differences in trait mindfulness were related to functional connectivity in the SN. Thirty-two undergraduate students completed the Mindfulness Awareness and Attention Scale (MAAS) on a separate testing session and underwent a 7-minute resting state functional magnetic resonance imaging scan. An independent components analysis was conducted to identify and create functional connectivity maps of the SN. Participants' scores on the MAAS were entered as a covariate to determine how mindfulness influenced functional connectivity within the brain's SN. High MAAS scores were associated with increased functional connectivity in the bilateral anterior and posterior insula and bilateral ACC as well as decreased function connectivity in the precuneus. These findings suggest high trait mindfulness enhances functional connectivity in key nodes of the SN while at the same time decreasing connectivity in a key node of the default mode network (DMN). This is especially interesting as the DMN is associated with mind wandering. These results follow the underlying principles of mindfulness, suggesting this relatively simple mindset is beneficially associated with alterations in the neural pattern of resting state functional connectivity.

Disclosures: **E.M. Bilevicius:** None. **S.D. Smith:** None. **J. Kornelsen:** None.

Poster

555. Perception and Imagery

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Program#/Poster#: 555.14/MMM4

Topic: H.02. Human Cognition and Behavior

Title: Neural correlates of self-criticism and self-praise in healthy participants and their relation to mindfulness and depression

Authors: ***J. LUTZ**[1], U. HERWIG[2], A. BRÜHL[2];
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Abstract: Self-criticism is a vulnerability factor for depression. The processing of self-criticism encompasses activation in self-related cortical midline regions, emotion-generating regions, such as the amygdala, along with prefrontal regulatory activations in healthy subjects. In contrast, neural and behavioural correlates of self-praise and their relation to depression is less well understood. At the same time, mindfulness, the non-judgemental awareness of experience in the moment, potentially influences the perception and regulation of such self-related emotions. Twenty-three healthy participants (11 women, 12 men, M_{age} = 44.4, *SD* = 11.4) were presented with previously chosen, and thus individually tailored stimuli of self-praise, self-criticism, negative but not self-related trait adjectives and neutral adjectives in a block-designed fMRI experiment. Compared to neutral stimuli, individualized self-criticism and self-praise activated similar frontal and emotion processing areas. Correlation analyses further revealed that people with high mindfulness scores showed decreased DLPFC activity, both during self-criticism and self-praise. Activations during self-criticism but not during self-praise were related to symptoms of depression. Our results suggest that most activation patterns previously reported for self-criticism are present during self-praise and therefore seem to be valence-unspecific. Further, individuals with higher trait mindfulness seems to recruit fewer regulatory resources when facing self-related emotions. Finally, we confirm the clinical relevance of self-criticism for depression, while positive self-appraisals might be more related to measures of resilience.

Disclosures: J. Lutz: None. **U. Herwig:** None. **A. Brühl:** None.

Poster

555. Perception and Imagery

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 555.15/MMM5

Topic: H.02. Human Cognition and Behavior

Support: National Committee of Science and Technology of Chile (CONICYT), through a doctoral scholarship given to Baquedano C (number 1070761)

Title: A brief mindful instruction de-automatizes approach-oriented impulses towards attractive foods: Behavioral and ERP evidence.

Authors: C. BAQUEDANO[1,2,3], V. LOPEZ[1], C. FABAR[1], *D. COSMELLI[1], A. LUTZ[2,3]; [1]Escuela de Psicologia, Pontificia Univ. Católica de Chile, Santiago, Chile; [2]Lyon Neurosci. Res. Center, INSERM U1028, CNRS UMR5292, Lyon, France; [3]Lyon 1 Univ., Lyon, France

Abstract: Being aware that mental events are mere representations and not necessarily an accurate depiction of reality, is one of the key feature of mindfulness meditation. In Western psychology this process has been called decentering, de-reification or cognitive diffusion. Decentering can be opposed to experiential fusion, the process of being lost or totally immersed into the contents of one's mind. Excessive experiential fusion is a hallmark in the prediction of psychological distress, and of several psychiatric disorders such as depression or addictive disorders .

Here we investigated whether a "decentered attitude" compared to an "immersed attitude", as induced by brief instructions, differentially modulate automatic approach-avoidance tendencies when processing visual stimuli. Specifically, we focused on how these instructions modulated impulses towards attractive foods.

In a paradigm adapted from Papies et al 2012, 50 healthy participants saw first neutral and attractive food pictures with either a mindful or immerse attitudes. Then participants performed an Approach-Avoidance Task (AAT) based on these images[7]. Dependent measures include behavioral and salivary volume measures, electroencephalography (EEG, 64 channels), self-report questionnaires, and qualitative interviews.

Replicating Papies et al. 2012, we found reduced automatic bias toward attractive food only in visual stimuli that followed the mindful instruction but not the immersed instruction, as measured by performances in the ATT. We also found that the saliva volume was reduced during the mindful condition compared to the immersed one and that the two instructions differentially impacted N1 early (80ms-120ms) evoked related potentials (ERPs) during AAT.

Overall, these findings suggest that a decentered attitude de-automatizes the approaching bias towards attractive stimuli found during experiential fusion and that this is given by a modulation in an early stage of image processing during the Approach-Avoidance conflict. These findings provide novel information on the mechanisms by which mindfulness-based interventions for food disorders or addiction could strengthen the participants' cognitive capacity to down-regulate craving.

Disclosures: **C. Baquedano:** None. **V. Lopez:** None. **C. Fabar:** None. **D. Cosmelli:** None. **A. Lutz:** None.

Poster

555. Perception and Imagery

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 555.16/MMM6

Topic: H.02. Human Cognition and Behavior

Title: Phenomenoconnectomics

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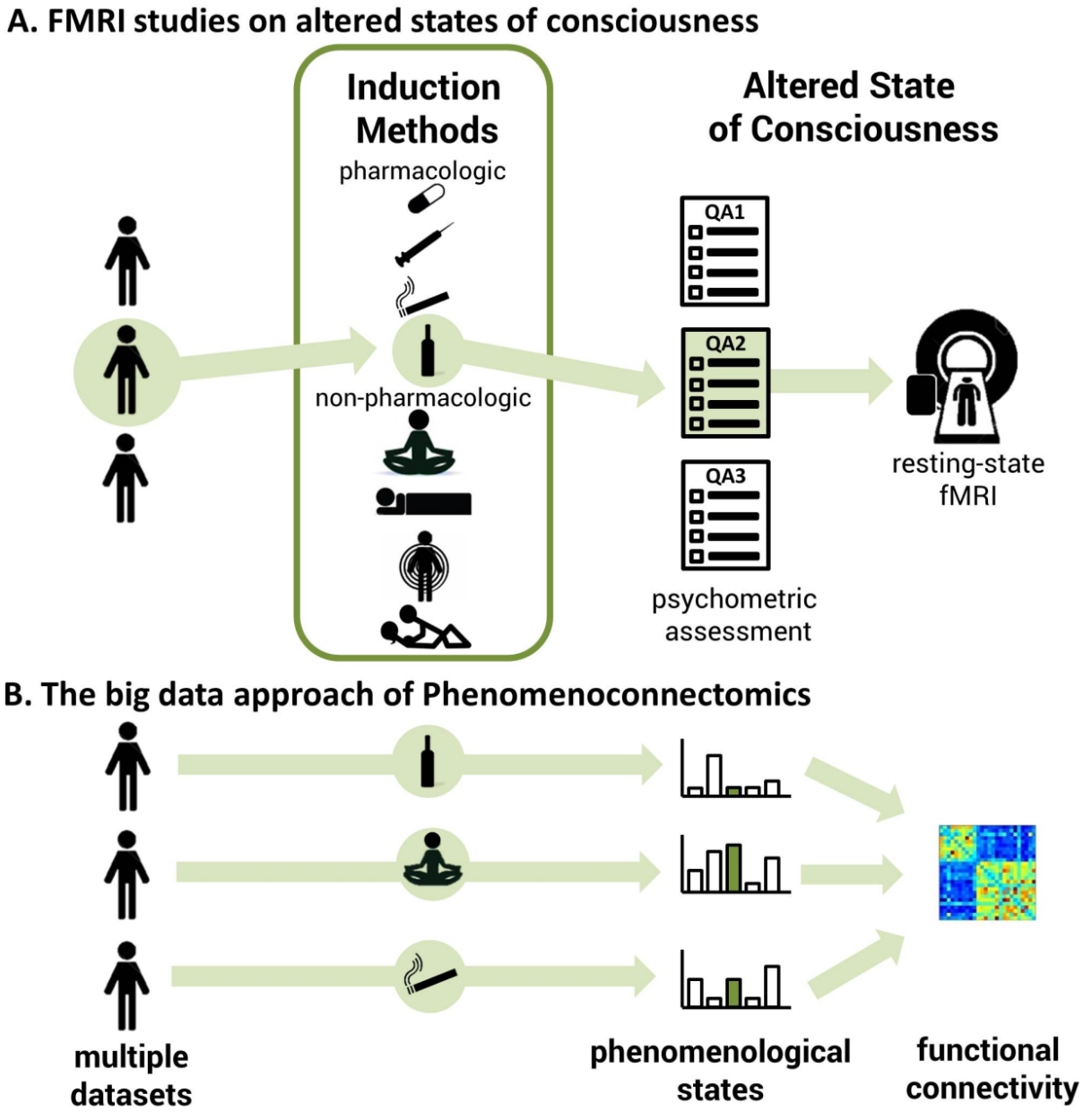
Abstract: *Phenomenoconnectomics* is a new empirical approach to relate phenomena of subjective experiences to modulations of brain connectivity. Analogous to other Big Data approaches it relies on the joint analysis of data from multiple neuroimaging studies.
Inducing altered states of consciousness (ASCs) within neuroscientific experiments aims to characterize brain mechanisms underlying changes in perception and thinking. Multiple recent fMRI-studies reported changes in functional brain connectivity when psychoactive substances such as LSD were administered. Besides pharmacological treatments, numerous other induction methods for ASCs are available that can be utilized in human research (e.g. meditation- or breathing-techniques). The careful assessment of the subject's phenomenological state with standardized psychometric tools allows the study of similarities in ASCs induced using different techniques. However, individual neuroimaging studies on ASCs are limited to small numbers of subjects. They therefore suffer from low statistical power and often do not allow to map the variability in subjective experiences to underlying brain processes.
Phenomenoconnectomics aims to overcome these limitations by the joint analysis of data from multiple ASC studies. The analysis of changes in functional connectivity in relation to ASC phenomena (e.g. Hallucinations) will allow descriptions such as: "When subjects experience X, the brain network Y exhibits the connectivity pattern Z, independent of how X was induced." Here, I will present the newly developed Altered States of Consciousness Database (ASDB), as the technical framework for *Phenomenoconnectomics*, by providing: (1) an overview on experimentally applicable induction methods for ASCs in humans, (2) a pool of psychometric assessment tools for subjective experiences, (3) meta-analyses on psychometric data for different induction methods of ASCs (years 1960-2016), (4) the computational framework for a Big Data approach to identify changes in functional connectivity patterns related to experiences of ASCs.



A. Multiple recent resting-state fMRI studies explored effects on brain connectivity, when altered states of consciousness (ASC) were induced. Several studies were reported using pharmacological as well as non-pharmacological induction methods (e.g. Trance, Hypnosis). The possibility to identify brain networks that correlate with specific ASC phenomena (e.g. hallucinations, ego disolution, fear etc.) within individual studies are limited, due to induction method specific effects. **B.** The approach of **Phenomenoconnectomics** combines multiple ASC neuroimaging datasets for joint meta-analyses. It allows to dissociate particular phenomena that appear accross differently induced ASCs. The standardized assessment of such phenomena with psychometric tools (questionnaires) allows to relate them to changes in brain connectivity.

Disclosures: T. Schmidt: None.

Poster

555. Perception and Imagery

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Program#/Poster#: 555.17/MMM7

Topic: H.02. Human Cognition and Behavior

Support: a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI16C0132)

Title: Effect of delirium Notices on Clinical Outcomes: Do you know your patient in delirium?

Authors: *J. PARK[1,2,3], J. PARK[1,2,3], M.-K. KIM[1,2,3], J.-S. HEO[3,4], J. AHN[3,5], S. PARK[6], W.-J. CHOI[3,7];
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Abstract: Background: The incidence of delirium is high in ICU as 20~40%, and it is a risk factor to increase the duration of a hospital stay, morbidity, and mortality. However delirium tends to have been overlooked compared to other medical and surgical conditions, because it is thought to be a vitally ‘real’ emergency state or it is difficult to notice the delirium if not in specialized care. The purpose of this study is to exam the effect on clinical outcomes of the delirium notification system. Methods/Design: Since September 12, 2014, our team has performed the “Delirium and Distress Management Project: IDDM Project” to the ICU patients. In this project the psychiatrist evaluated the patients' delirium, anxiety, and pain daily. Since December 1, 2014 the psychiatrists have noticed the delirium state by two methods. One is to hang the sign on the bedside of the patient and the other is to have the warning sign in the electronic medical chart appear. The preliminary analysis of the data was January to March 2014,before operating notice systems were in use, and January to March 2015 after operating notice systems were in use. Result: The severity of medical illness score was higher in 2015 (mean=14.9, SD=10.0) than 2014 (mean 12.5, SD=7.9). Under controlling it as confounding factor, the incidence of delirium, hospital day, and mortality were not different between the two periods. The pain score was showed a statistically significant decrease after the notice than before the notice (2014: mean 2.68, SD 2.35, 2015: mean 2.33, SD 0.80). The anxiety score was also diminished in 2015 (mean 11.29, SD 5.55) compared to 2014 (mean 12.32, SD

5.60).Conclusion The result showed improvement of the clinical outcome of pain and anxiety. Considering the pain and anxiety are the significant factor for affecting delirium, the proper intervention of them might help control the delirium. Our team has been analyzing bigger data for illumination on this subject, and expect more insight on this issue.

Disclosures: **J. Park:** None. **J. Park:** None. **M. Kim:** None. **J. Heo:** None. **J. Ahn:** None. **S. Park:** None. **W. Choi:** None.

Poster

556. Human Learning: Spatial

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Program#/Poster#: 556.01/MMM8

Topic: H.02. Human Cognition and Behavior

Support: NSERC #239896-2013

CIHR

Title: Patients with medial orbitofrontal lesions show reduced spatial memory

Authors: ***L. DAHMANI**[1], Y. YANG[1], L. K. FELLOWS[2], V. D. BOHBOT[3];
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Abstract: Aim: In a previous study, we found that spatial memory was associated with increased fMRI BOLD activity and grey matter in the medial orbitofrontal cortex (mOFC). To further determine the critical contribution of this region in spatial memory, we investigated spatial memory in patients with mOFC lesions.
Methods: We tested 20 control participants and 16 patients with brain damage to the frontal cortex: 8 patients with mOFC (mOFC+ group) and 8 patients with frontal lesions that did not overlap with the mOFC (mOFC- group). Participants were administered the 4-on-8 virtual maze. In the 4-on-8 virtual maze, participants have to learn the location of 4 target objects among 8 arms. Then, a probe trial is administered in which landmarks are taken away. The probe gives an indication of how much participants relied on landmarks to find the objects and gives an index of spatial memory, as landmark use is a hallmark of the spatial memory strategy. Finally, we administered the Rey Auditory Verbal Learning Test (RAVLT) and the Test of Nonverbal Intelligence-3 (TONI-3) to investigate verbal memory and IQ. We compared the three groups on their navigation strategies as well as their performance on the navigation task and neuropsychological tests.

Results: The mOFC+ group performed better than the control group on the probe trial of the 4-on-8 virtual maze, indicating that they relied less on landmarks. Verbal reports following testing revealed that the mOFC+ group noticed fewer landmarks in the environment than the other two groups. The mOFC- group, on the other hand, showed deficits in learning, regardless of navigation strategies. The mOFC- group also performed worse than the control group on the RAVLT and had lower IQ scores than the other two groups.
Conclusion: We had previously found that spatial memory was associated with increased fMRI BOLD activity and grey matter in the mOFC. In the current study, we confirm the critical contribution of this area as patients with lesions to the mOFC show reduced spatial memory without showing global cognitive deficits. On the other hand, the learning deficits exhibited by the mOFC- group may be due to global cognitive deficits.

Disclosures: **L. Dahmani:** None. **Y. Yang:** None. **L.K. Fellows:** None. **V.D. Bohbot:** None.

Poster

556. Human Learning: Spatial

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Program#/Poster#: 556.02/MMM9

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant P01-AG017586

Title: Perceptual generalization and category induction are compromised in Alzheimer's disease and semantic dementia

Authors: ***J. S. PHILLIPS**, C. T. MCMILLAN, M. GROSSMAN;
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Abstract: Learning of semantic concepts depends on the ability to generalize across details of learning episodes. Low-level generalization requires abstraction from specific episodic details to recognize semantically related but perceptually novel instances of a category. Higher-level generalization involves recognition of supraordinate category relationships between subcategories. We used a visual category learning task to examine perceptual generalization of learned features and the ability to form a new supraordinate category representation. Participants were older adults with normal cognition (n=11), semantic dementia (SD; 6), Alzheimer's disease (AD; 9), or mild cognitive impairment (MCI; n=8), a prodromal phase of AD. Materials were images of fictitious animals that varied on 7 features (e.g., head shape, horns). We hypothesized patients would be impaired relative to controls in all aspects of learning. SD patients were expected to perform worst due to selective anterior temporal lobe atrophy, with lesser deficits for

AD patients, and least impairment in MCI. In Phase 1, participants viewed items from 2 distinct categories ("X" and "Y") one at a time. They matched each item to a model image for the correct category and received immediate feedback. In Phase 2, participants had to categorize perceptually novel exemplars of X and Y categories. Additionally, they had to reject items of a new, third category ("Neither"). Perceptual generalization was assessed as the difference in X and Y accuracy between Phases 1 and 2. Accuracy was highest for controls, followed by MCI, SD, and AD patients. However, controls and MCI patients had decreased accuracy for perceptually novel items, suggesting that initial rejection of perceptual novelty was part of normal generalization. In Phase 3, participants were told that X and Y items should be considered members of a single category, and they should discriminate the joint X/Y category from the Neither category. Category induction was assessed by participants' d-prime scores. Controls discriminated best between Neither and supraordinate X/Y categories, followed by MCI, SD, and AD patients. Results confirmed that perceptual generalization and new category induction are relatively preserved in MCI, but are compromised in SD and AD.

Disclosures: **J.S. Phillips:** None. **C.T. McMillan:** None. **M. Grossman:** None.

Poster

556. Human Learning: Spatial

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Topic: H.02. Human Cognition and Behavior

Support: CIHR Grant 86727

CIHR Grant 82638

CIHR Grant 301763

Title: Entorhinal cortical atrophy in asymptomatic APOE4 carriers at risk for Alzheimer's disease

Authors: ***K. KONISHI**[1], R. JOOBER[1], J. POIRIER[1], K. MACDONALD[1], M. CHAKRAVARTY[2], R. PATEL[2], J. BREITNER[1], V. D. BOHBOT[1];
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Abstract: Early detection of Alzheimer's disease (AD) has been challenging as current biomarkers, such as neuroimaging, and measures of amyloid and TAU, are invasive and costly. Strong predictors of future AD diagnosis include lower volume of the hippocampus and

entorhinal cortex. In addition, the ε4 allele of the Apolipoprotein E gene (APOE) gene is the strongest known genetic risk factor for AD. These studies suggest that studying functions that are critically mediated by the hippocampus and entorhinal cortex, such as spatial memory, in APOE-ε4 allele carriers, may be key to the identification of individuals at risk of AD, prior to the manifestation of cognitive impairments. Using a virtual navigation task developed in-house, specifically designed to assess spatial vs non-spatial compensatory strategies, the current study is the first to differentiate APOE-ε4 allele carriers with atrophy in the hippocampus, entorhinal cortex and fimbria from APOE-ε4 allele carriers with grey matter comparable to non-APOE-ε4 allele carriers.

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Poster

556. Human Learning: Spatial

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Topic: H.02. Human Cognition and Behavior

Support: Korea NRF Grant 2015R1D1A1A01059743

Title: Task difficulty effects on object-location binding memory performance according to gender difference

Authors: ***J. PARK**[1,1], T. KIM[1], Y. M. PARK[1], S. I. KIM[1], I. Y. KIM[1], J. K. KANG[2], J.-J. KIM[3], D. P. JANG[1];
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Abstract: Research on sex differences in spatial cognition has reported that there are distinct tests for male-favoring or female-favoring performance. There were sex differences in object-location binding memory performances according to stimulation type and measurement parameters. To investigate the inconsistent results obtained regarding sex differences in object-location memory, we used the fractal objects version of the 'What was where?' task developed by Pertzov et al. to measure object measurement and location memory performance in object-location memory under conditions that reduced the use of verbal strategies. In particular, object-location memory performance was measured by 'swap error'. The 'swap error' is defined as when participants relocate target objects around the locations of other presented objects rather

than at random locations. This 'swap error' is not a simple failure of object identity or object location, but rather indicates failure to bind the object identity and object location in memory. Therefore, this measurement is more sensitive for object-location binding memory performance. The purpose of this study is to investigate sex differences in object-location memory by controlling task difficulty and using more sensitive measurements, including object memory, location memory and object-location binding memory. Females outperformed males on 'swap error' measurement, where an object-location binding memory index was calculated based on the number of times the participants confused an object's location with that of another object. One of our most interesting findings is that the swap error of males increased at a higher rate in difficult sessions compared to females. When only the four-object trials from Session 34 and Session 45 were compared, in which the level of difficulty was presumed to be similar but with varying mental load, the 'swap error' of males was significantly increased in Session 45 compared to females. In conclusion, there may be gender differences in object-location binding memory and the binding memory performance of males could be influenced more by mental or cognitive load than that of females.

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Poster

556. Human Learning: Spatial

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Program#/Poster#: 556.05/MMM12

Topic: H.02. Human Cognition and Behavior

Support: CIHR Grant 301763

Title: Modulation of spatial and response strategies by phase of the menstrual cycle in women tested in a virtual navigation task

Authors: *V. D. BOHBOT[1], D. HUSSAIN[2], S. HANAFI[3], K. KONISHI[3], W. G. BRAKE[2]; [1]Douglas Mental Hlth. Univ. Institute, Dept. of Psychiatry, McGill Uni, Verdun, QC, Canada; [2]Psychology, Concordia Univ., Montreal, QC, Canada; [3]Psychiatry, Douglas Mental Hlth. Univ. Institute, McGill Univ., Verdun, QC, Canada

Abstract: Different memory systems are employed to navigate an environment. It has been consistently shown in rodents that estrogen impacts multiple memory system bias such that low estradiol (E2) is associated with increased use of a striatal-mediated response strategy whereas high E2 increases use of a hippocampal-dependent spatial memory. Low E2 also enhances

performance on a response-based task whereas high E2 levels improve learning on a spatial task. The purpose of the present cross-sectional study was to investigate navigational strategies in young, healthy, naturally cycling women. Participants were split into either an early follicular (i.e., when E2 levels are low), ovulatory (i.e., when E2 levels are high) or mid/late luteal (i.e., end of the cycle, when E2 levels decrease and progesterone levels rise) phase group, using self-reported date of the menstrual cycle. Serum hormone level measurements (E2, progesterone, testosterone) were used to confirm cycle phase assignment. Participants were administered a verbal memory task as well as a virtual navigation task that can be solved by using either a response or spatial strategy. Women tested in the ovulatory phase, under high E2 conditions, performed better on a verbal memory task than women tested during the other phases of the cycle. Interestingly, women tested in the mid/late luteal phase, when progesterone is high, predominantly used a spatial strategy, whereas the opposite pattern was observed in the early follicular and ovulatory groups. Our data suggest that the specific memory system engaged differs depending on the phase of the menstrual cycle and may be mediated by both E2 and progesterone, rather than E2 alone.

Disclosures: **V.D. Bohbot:** None. **D. Hussain:** None. **S. Hanafi:** None. **K. Konishi:** None. **W.G. Brake:** None.

Poster

556. Human Learning: Spatial

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Support: NIH/NEI Grant R01 EY021818 (F.A.M.)

Core Grant P30EY022589

Frontiers of Innovation Scholars Program

Qualcomm Institute CSRO 140 Grant

Title: Virtual environment human navigation task for assessing spatial cognition and navigation

Authors: ***C. STEVENSON**[1,2,3], A. ELHOSSEINY[4,5], A. DINIZ-FILHO[5], G. CAUWENBERGHS[1,2], F. MEDEIROS[5], E. MACAGNO[3,6];
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Abstract: In order to perform the wayfinding necessary for efficient navigation, individuals must observe their environments, remember positions and properties of multiple features, and integrate them into a usable representation of the world. Various conditions can detrimentally affect the ability to perform such tasks, including loss of visual acuity or cognitive impairment. It is important to understand how these conditions affect spatial cognition and how we can design spaces to improve individuals functions, particularly as they age.
To study this we have developed the Virtual Environment Human Navigation Task (VE-HuNT). This is an experimental paradigm which uses virtual reality to construct a simulated 3D immersive environment and related experimental tasks to assess various aspects of spatial cognition. VE-HuNT is run on the 4kave, a more cost-effective derivative of the immersive CAVE VR systems, providing a realistic, yet controlled virtual environment within which to perform experiments. VE-HuNT allows researchers to implement a variety of cognitive tasks within a virtual room (or rooms), while monitoring subject progress in real time.
We used VE-HuNT to assess the effects that environmental cues had on individuals abilities to navigate and how subjects learned. Seated subjects used a steering wheel and pedal to move through three virtual rooms, each with a different complement of spatial cues. In each room, the subject was shown a target on the floor somewhere in the room and instructed to move to it. The target was then hidden from view and the task repeated, finding the same location from different start points. We recorded temporal and positional information and used the path data to analyze the abilities and strategies subjects employed to complete the tasks. Traditional metrics of visual and cognitive function were also collected.
From this data we were able to assess the impact that environmental conditions had on path learning, strategies in navigation, and how these were affected by sensory deficits. It was shown that subjects learned the location of the target in each room. Whether or not cues were spatially precise and their relative positions to each other had an effect on the time subjects took to find the target. This effect became more pronounced in subjects with significant visual loss, due to advanced glaucoma. Subjects were also found to use various strategies for finding their location within the room, including visually scanning the room before moving and/or moving to a particular location in the room before proceeding to the target.

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Poster

556. Human Learning: Spatial

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Program#/Poster#: 556.07/MMM14

Topic: H.02. Human Cognition and Behavior

Support: R01-MH062500

Title: Using virtual reality environments to assess context segmentation and spatial memory performance using a 2d and virtual reality test environment.

Authors: *K. M. HORECKA[1,2], M. R. DULAS[3], C. WIDDOWSON[3], N. J. COHEN[3];
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Abstract: Virtual Reality (VR) has been identified as a useful tool in neuropsychological evaluation thanks to its increased control and measurement capabilities over other methods (Schultheis et al, 2002). More recently, evidence shows that along many measures, VR provides similar ecological validity to real environments (Kuliga et al, 2014). One area of research which is particularly amenable to VR paradigms is learning and memory. Previous work has suggested that context-boundaries impact the binding of sequential information, resulting in within-context information being remembered as closer together than across-context information, even when equidistant, i.e. the segmentation effect (DuBrow & Davachi, 2013). The present study explored segmentation effects in spatial memory; a VR spatial navigation task with four contexts (colored rooms) and 4 objects per context was constructed to evaluate how memory for item pair locations within a context differ from pairs which cross contexts. During study, participants were instructed to explore the VR environment (beginning from pseudo-random positions and orientations) and learn locations of all items before being tested on both 2D map and in the VR environment. Measurements of participant position, orientation, and memory for item locations were assessed across four trials. Results showed significant increases in performance (speed, efficiency of movement/orientation, and memory accuracy) across successive training/test trial, as well as a significant difference in memory for the distance between within-context and across-context item pairs, with across-context pairs being remembered as further apart and within-context pairs closer together despite these pairs being equidistant). Additionally, participants showed larger segmentation effects in the 2D testing paradigm than VR. Finally, across all trials, segmentation effects remained relatively stable in VR test, while 2D test showed a significant linear decrease in across-context distances across the trials. These results show that virtual reality can be used successfully in exploring spatial memory and segmentation stability across multiple trials, as well as provide tantalizing new measures which increased spatiotemporal resolution of behavior beyond other existing methods.

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Poster

556. Human Learning: Spatial

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Topic: H.02. Human Cognition and Behavior

Support: UCLA Startup Funds

DARPA restoring active memory program N66001-14-2-4029

Title: Theta oscillations in the human medial temporal lobe during ambulatory movement

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Abstract: Theta oscillations are implicated to play a critical role in learning and memory by coordinating the spiking activity of neuronal ensembles, via mechanisms such as spike timing dependent plasticity. This rhythm has been heavily studied in rodents, where it is continuously present during movement at frequencies within 6-12Hz. In humans, however, a functionally similar theta is thought to occur at lower frequency ranges (3-7Hz) and in shorter bouts. This lower frequency theta rhythm is observed during a variety of behaviors, including virtual navigation (1), but has never been tested during real world ambulatory movement.To examine the oscillatory properties within the human medial temporal lobe (MTL) during movement, we measured hippocampal and entorhinal intracranial EEG (iEEG) from 3 patients with pharmacoresistant epilepsy (two sighted and one congenitally blind) who are chronically implanted with the FDA approved NeuroPace RNS® Neurostimulator. Subjects performed a task in which they walked, following linear and circular paths at various speeds. Positional data was tracked at 60Hz using the Optitrack system with sub-millimeter motion tracking; and iEEG was recorded at 250Hz and stored in the RNS Neurostimulator with an analogue filter equivalent to a 1st order Butterworth with 3db attenuation at the cutoff frequencies (4-90Hz). Electrode localization was performed through co-registration of a high-resolution post-operative CT and high-resolution pre-operative MRI. To characterize significant oscillations within different

frequency ranges, we used the BOSC detection algorithm (2). Episodes with significant oscillations between 3-20Hz (occurring for at least 3 cycles and above 95% chance level) were detected and the percentage of time during which they were present was computed for low and high movement speeds (divided based on the median speed). We found that in all 3 subjects, there was a significant increase in this measure in the theta range typically associated with rodent movement (6.5-9.5Hz, $P<0.05$, $N=72$) during fast movements compared to slow movements. In the sighted patients, these episodes were transient and present ~30% of the time, while this percentage was ~65% in the visually-impaired subject. Our results suggest that higher frequency theta indeed exists in humans during movement. The precise link between this pattern and its behavioral correlates will be an exciting area for future study given this novel methodology for simultaneous motion capture and iEEG recording during ambulatory human behavior.

1. A. J. Watrous *et al.*, *Hippocampus*. **23**, 656-61 (2013).
2. A. M. Hughes *et al*., *Hippocampus*. **22**, 1417-28 (2012).

Disclosures: Z. M. Aghajan: None. **P. Schuette:** None. **T. Fields:** None. **M. Tran:** None. **N. Hasulak:** None. **T. Tcheng:** None. **D. Eliashiv:** None. **J. Stern:** None. **I. Fried:** None. **N. Suthana:** None.

Poster

556. Human Learning: Spatial

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G. Harold & Leila Y. Mathers Foundation (09212007)

Title: Theta-burst microstimulation in the human entorhinal area improves memory

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Abstract: Restoration of memory function using electrical stimulation to modulate activity in the human medial temporal lobe has recently become a focus of considerable interest and stimulating targets such as the fornix[1] and the entorhinal area[2] have shown promise for memory improvement. However, these studies were conducted by applying high amplitude currents via large electrode contacts, which provide limited regional specificity. In this study, we applied low-current 150 μA electrical stimulation in a theta-burst pattern (4 100 Hz pulses at 5 Hz) through 100 μM micro-electrodes to the entorhinal area while participants completed a face recognition memory task.

Patients with pharmacoresistant epilepsy who met clinical criteria were implanted with depth-electrodes in the hippocampus and the entorhinal area for seizure localization. Patients learned an average of 49±7 novel portraits of people on a computer screen during an encoding phase where the number of learned images was based on subject-specific neuropsychological memory scores. Following a distractor task, the same images from the encoding phase (targets) and similar-looking portraits of different people (lures) were presented during a retrieval phase. In the retrieval phase, patients were asked to identify whether the image was NEW or OLD via button press. Trials were classified as *recollected* if the subject correctly identified the target ***and*** correctly rejected the corresponding lure. The right entorhinal area (REA) or the left entorhinal area (LEA) was stimulated during learning preceding half of the trials in a randomized fashion. Memory performance during stimulation and non-stimulation trials was then calculated in 9 patients (N = 9; REA, N = 5; LEA, N = 5) and 16 sessions (n = 16; REA, n = 8; LEA, n= 8). We used the Generalized Estimating Equations (GEE) to model the effects of stimulation and the side of the brain stimulated (REA or LEA) on the number of portraits recollected. Across all trials, the interaction between stimulation condition and the site of stimulation was statistically significant (GEE, $p=0.002$). There was a statistically significant effect of stimulation of the REA on number of pictures recollected (GEE, $p<0.0001$). However, these differences between stimulation and non-stimulation trials were not significant in the LEA for the number of pictures recollected (GEE, $p=0.491$). Taken together, our results show that theta-burst microstimulation of the REA during learning enhances subsequent recollection of novel portraits.

1. Hamani, C., et al. Ann Neurol, 2008. **63**(1): p. 119-23.
2. Suthana, N., et al. N Engl J Med, 2012. **366**(6): p. 502-10.

Disclosures: A.S. Titiz: None. **M.R.H. Hill:** None. **D. Eliashiv:** None. **N. Tchemodanov:** None. **U. Maoz:** None. **J. Stern:** None. **M. Tran:** None. **E. Mankin:** None. **E. Behnke:** None. **N. Suthana:** None. **I. Fried:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NINDS Grant NS084017

DARPA Restoring Active Memory Program N66001-14-2-4029

Title: Stimulation of entorhinal white matter enhances declarative memory encoding

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Abstract: The medial temporal lobe (MTL), which includes the hippocampus and adjacent entorhinal, perirhinal, and parahippocampal cortices, plays a critical role in the formation of declarative memories (*1*). Intracranial electrical stimulation has shown potential promise for improving hippocampal-dependent memory (*2*), yet little is known about how targeting different areas within the MTL could affect behavior. In this study we sought to determine the specific sites for which such stimulation can improve memory. Based on the functional circuitry of the hippocampal region, we hypothesized that stimulation of the entorhinal white matter during learning would generally improve subsequent memory while adjacent gray matter MTL stimulation during learning would, in fact, impair subsequent memory. Twenty-three patients (*M* age ± *SD*: 34.26 ± 12.34 yrs, 9 female) with pharmacoresistant epilepsy who had been implanted with depth electrodes for clinical evaluation participated in five different declarative memory tasks. High-resolution magnetic resonance imaging (MRI, (*3*)) and automated image segmentation methods (*4*) were used to localize 40 distinct intracranial stimulating electrodes within the MTLs of our participants to entorhinal white matter or adjacent MTL gray matter regions. A performance index was calculated as the percentage difference score for stimulated compared to non-stimulated trials, averaged across all memory tasks for a given electrode region. A positive performance index indicated that memory for stimulated trials was better than non-stimulated trials, while a negative performance index indicated that memory for non-stimulated trials was better than stimulated trials. Our results show that memory performance with white matter stimulation was significantly better than that with gray matter stimulation (N_{gray} = 16; N_{white} = 24; gray *Mdn*, [25th, 75th]: -5.80, [-21.48, 4.06]; white *Mdn*, [25th, 75th]: 20.05, [2.74,

50.72]; $p = .007$, $Z = 2.72$). Additionally, white matter stimulation significantly improved memory ($p = .01$, $Z = 2.58$), while gray matter stimulation showed no statistically significant memory effect ($p = .19$, $Z = -1.31$). The results suggest that stimulation of the entorhinal white matter, presumably the perforant path input to the hippocampus, can enhance declarative memory encoding, while stimulation of neurons in nearby gray matter is ineffective in improving MTL memory function.
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2. N. Suthana *et al. N Engl J Med* **366**, 502-10 (2012).
3. A. D. Ekstrom *et al. Neuroimage* **47**, 42-49 (2009).
4. P. A. Yushkevich *et al. NeuroImage* **53**, 1208-24 (2010).

Disclosures: P.J. Schuette: None. **M. Tran:** None. **A. Titiz:** None. **N. Tchemodanov:** None. **E.A. Mankin:** None. **Z.M. Aghajan:** None. **D. Eliashiv:** None. **J. Stern:** None. **S.A. Weiss:** None. **D. Kirsch:** None. **B. Knowlton:** None. **I. Fried:** None. **N.A. Suthana:** None.

Poster

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Program#/Poster#: 556.11/MMM18

Topic: H.02. Human Cognition and Behavior

Support: NARSAD

Seton Research Funding

Title: Grid cell activity in the human entorhinal cortex scales with the size of virtual environments

Authors: *Z. NADASDY[1,2,3], P. NGUYEN[4], Á. TÖRÖK[5], D. BRIGGS[6,8], J. SHEN[10], P. MODUR[6,9], R. J. BUCHANAN[7,9];
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Abstract: The activity of grid cells in the entorhinal cortex of the rodent, primate and human provide a spatial coordinate system that can inform an individual of its location. Grid geometry

in rodents is generally preserved across settings, but has displayed a limited degree of adaptation to certain environmental features. It is possible that the environment's influence on grid geometry varies across species and with evolution. Human subjects implanted with entorhinal cortical electrodes performing virtual navigation tasks enabled us to investigate this in humans, where the environment-grid relationship has not been studied. Here, we report that human entorhinal cortical neurons exhibit changes in grid period, grid geometry, and grid orientation in close association with changes in environment size, shape, and visual cues. Our results demonstrate that the human entorhinal cortex is endowed with an extensive adaptive capacity to incorporate context-dependent factors into its neuronal representations of space.

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Poster

556. Human Learning: Spatial

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Topic: H.02. Human Cognition and Behavior

Title: Weaving in and out of sight: RSC and OPA integrate the current field of view with the remembered 360-degree panorama.

Authors: *C. E. ROBERTSON[1], A. MYNICK[2], K. HERMANN[3], D. KRAVITZ[4], N. KANWISHER[3];
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Abstract: How are discrete and fleeting views of a panoramic environment knit into a unified representation in the brain? Here, we probed the psychological and neural mechanisms supporting panoramic visual memory (behavior and fMRI).
Participants were introduced to novel 360° panoramic environments: photospheres of real world locations, either dynamically revealed across a panoramic display or actively explored using a virtual reality headset from a naturalistic, egocentric perspective.
Viewing experience was controlled so that participants viewed either: 1) a contiguous, coherent panoramic scene (two overlapping quarters), 2) a discontiguous panoramic scene (two non-overlapping quarters of the same panorama), or 3) a contiguous, incoherent panoramic scene (a spliced panorama that smoothly morphed between two panoramic scenes).
Exp. 1 (behavior, n=20): We first demonstrate the conditions under which two discrete views from opposite poles of a 360° panoramic environment are associated in memory: non-

overlapping views of a visual environment are linked in memory only if the visual information standing between them is experienced, not by mere spatiotemporal co-occurrence ($p < 0.001$) or transitive association with a third, unrelated view ($p < 0.01$).
Exp. 2 (fMRI, n=12): These findings parallel neural changes: discrete views of a panoramic environment increase in representational similarity in two specific regions of the scene network, the RSC and OPA, as a function of visual memory for the spatial information that unites them (both $p < 0.003$). PPA did not evidence this effect, but did show robust scene- and view-selective responses ($p > 0.93$). These results demonstrate neural representations of the scene within the current field of view are imbued with our memory for the broader panoramic environment.
Exp. 3 (behavior, n=20): Finally, memory for a broad, panoramic environment causes discrete views from within this environment to prime each other in perception. On each trial, participants were shown an image from one of the studied panoramas and asked to remember its spatial position (left/right side of the panorama). The image was primed by either another image from the same panorama (Valid Prime) or a black square (Neutral Prime). We observed significant perceptual priming between scene views from the same panorama (Valid - Neural), as a function of visual memory for their linking spatial information ($p < 0.006$).
Our findings demonstrate that individual perceptual experiences trigger visual memory for the panoramic environment far beyond the current field of view - both in the brain and in perception - helping to support our sense of a seamless panoramic visual expanse.

Disclosures: C.E. Robertson: None. **A. Mynick:** None. **K. Hermann:** None. **D. Kravitz:** None. **N. Kanwisher:** None.

Poster

556. Human Learning: Spatial

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Topic: H.02. Human Cognition and Behavior

Title: Translational episodic and episodic-like memory models: paired associate learning vs. what-where-which

Authors: *R. F. LANGSTON, J. DUNCAN, E. MIDDLETON;
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Abstract: Episodic memory testing methods are inconsistent between humans and animals, between clinicians and psychologists, and between age groups being tested. We aim to determine the effects of the theoretical and procedural differences between episodic memory tests commonly used in humans and rodents and create novel standardised protocols that can be used

in both populations, in health and disease and throughout the lifespan. We designed two tests, one based on the clinically used dementia diagnostic Paired Associate Learning (PAL) and the other based on the rodent behavioural test of episodic-like memory, associative what-where-which(WWW) memory. The human test is traditionally screen based and the rodent test is traditionally done with 3D stimuli but we adapted both to a procedurally identical screen version. Participants' reaction times and number of errors in selecting stimuli were recorded. Initial experiments on 83 healthy student volunteers revealed a striking difference in memory performance between the clinically used paired associate learning test and the experimentally used episodic-like memory test. We then went on to study the factors which may govern this difference in difficulty. The presence (WWW) or absence (PAL) of an additional contextual component to the stimulus scene, stimulus presentation (either sequential or concurrent) and memory strategy (recollection or recognition) were identified as the main differences between the two tasks. 60 student volunteers then carried out eight different memory tests with the above variables incorporated in all their possible combinations. The presence of a contextual component (as in WWW) had a significant effect on performance with subjects accumulating more errors in the WWW than the PAL task in their original forms. Forcing the use of a recall strategy (as in PAL) rather than allowing recognition memory to be used (as in WWW) had no effect on performance in either task. The way in which stimuli were presented had a significant effect on performance. Sequentially presented stimuli (as in PAL) resulted in a greater number of errors than concurrent presentation (as in WWW). Participants also provided a confidence score after each response and completed tests at 2 different difficulty levels. Confidence score showed an inverse correlation with difficulty level but not with any other performance measures. Overall the results indicate that the WWW rodent episodic-like memory task is more difficult than the human PAL task, however scores on each did show a positive correlation indicating that they are somewhat comparable.

Disclosures: **R.F. Langston:** None. **J. Duncan:** None. **E. Middleton:** None.

Poster

556. Human Learning: Spatial

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Topic: H.02. Human Cognition and Behavior

Support: Princeton University CV Starr Fellowship

NIH R01 EY021755

Title: Reconstructing spatial location and forward planning during navigation

Authors: *J. W. ANTONY, C. BALDASSANO, M. ALY, K. A. NORMAN, N. B. TURK-BROWNE;
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Abstract: The spatial location of an animal can be reconstructed from the activity of neurons in the hippocampus and other brain regions. Similar regions have been implicated in human spatial processing, but relatively little work has been aimed at reading out exact spatial location. Here we use inverted encoding models applied to high-resolution fMRI data to reconstruct spatial location during both actual and planned navigation. We trained a model with 91 spatial channels that tiled a circular arena in a virtual reality environment while participants searched for a hidden platform. Although circular, the arena contained two visual landmarks on opposing walls that participants could use to orient themselves. Platform locations changed every second trial, forcing participants to search for the platform in an exploratory manner on odd-numbered ("unknown") trials, but allowing them to use memory for the platform location from the previous trial on even-numbered ("known") trials. Critically, this paradigm forced participants to fully explore the environment across trials, generating sufficient data to train the model. We trained voxel-wise weights for each channel using each participant's location data from most of the unknown trials and then performed cross-validation by using these weights to estimate participant location on the held-out trials. We found that spatial location could be reconstructed with above-chance accuracy in early visual cortex and the superior parietal lobule. Next, we asked whether and how participants represent the platform location during planning by applying the trained model to the known trials. We restricted analysis to timepoints during which they were located at the starting point of the environment, before starting to move. Because the location and view were constant across trials during these timepoints, above-chance reconstruction necessarily indicates that a region contains a memory representation of the platform location. In this analysis, we found reliable information about platform location in the left hippocampus. Further analyses examined other features of the task, such as heading direction/orientation and distance to the platform, and sought to quantify receptive field sizes for location and orientation.

Disclosures: J.W. Antony: None. **C. Baldassano:** None. **M. Aly:** None. **K.A. Norman:** None. **N.B. Turk-Browne:** None.

Poster

557. Motor and Sequence Learning

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Program#/Poster#: 557.01/MMM22

Topic: H.02. Human Cognition and Behavior

Title: Procedural learning with and without feedback are impaired by inhibition of the dorsolateral prefrontal cortex (DLPFC)

Authors: *L. WILKINSON, P. KOSHY, E. EWUL, E. M. WASSERMANN, S. SCHINTU; Natl. Inst. of Neurolog. Disorders and Stroke, NIH, Bethesda, MD

Abstract: Motor sequence learning is slowed by inhibitory TMS of the primary motor area (M1) contralateral to the performing hand, while motor function remains unchanged. This could be from interference with the reinforcement learning network of cortical and basal ganglia areas or with a network specific for motor learning. The right DLPFC is implicated in procedural learning on the non-motor Weather Prediction Task (WPT) and has its own basal ganglia link. If it supports procedural learning in general or provides access to such a network, inhibitory TMS there should also interfere with motor learning. Enhanced feedback with monetary reward and punishment speeds learning and should increase reliance on the reinforcement learning network. We delivered real continuous theta-burst TMS (cTBS; n= 15) or sham over right DLPFC (n= 15) or sham (n= 20) to two groups before they performed a motor sequence learning (serial reaction time) task in two different sessions, where they learned either with or without monetary reward/punishment, based on response accuracy and reaction time (RT). Subjects trained on two intermixed sequences: one more (85%, probable sequence), one less (15%, improbable sequence), likely to determine cue order. We measured learning as the difference in RT on probable and improbable sequence trials. In each session, subjects were tested for sequence recall 60 minutes after stimulation. Real cTBS impaired initial learning and sequence recall when the task was learned with and without feedback. Moreover, real cTBS abolished the usual improvement in learning seen with monetary reward/punishment. In an earlier study, we did not see this with cTBS delivered to M1. We conclude that the right DLPFC provides TMS access to a network involved in procedural learning under reinforcement conditions.

Disclosures: L. Wilkinson: None. **P. Koshy:** None. **E. Ewul:** None. **E.M. Wassermann:** None. **S. Schintu:** None.

Poster

557. Motor and Sequence Learning

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Topic: H.02. Human Cognition and Behavior

Support: FNS Grant 32003B-155947

Title: Resting state connectivity in parietal regions supports visuo-motor skill learning

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Abstract: Procedural learning, including learning of a visuo-motor skill, is subject to fast training-induced plasticity and to offline consolidation. Improvements are typically seen during the training itself, but also following learning as the skill continues to evolve offline during sleep. The present study investigates how brain connectivity relates to plastic changes during visuo-motor skill learning and to offline consolidation. Twenty healthy participants were assigned to one of two groups: The experimental group performed a computerized mirror-tracing task, in which right-left movements with the mouse were reversed on the screen. The control group performed a similar task but with concordant direction of cursor movements. Comparison of the two groups allows dissociating visuo-motor skill learning from simple motor performance. Participants trained for 15 minutes on Day 1. High-density EEG (156 electrodes) was recorded at rest for both groups before and immediately after training. Subjects were again tested for offline consolidation 24h later. The mirror-tracing group, but not the control group, showed greater tracing accuracy and faster completion time across trials in the training session. The mirror-tracing group, but not the control group, showed offline improvement from Day 1 (last 4 trials) to Day 2 (first 4 trials) in tracing accuracy (31% improvement) and completion time (27% improvement). Connectivity analyses showed increased coherence in the superior right parietal cortex in the alpha-beta range (8-20 Hz) from before to after training on Day 1 for the experimental group and decreased coherence in the same area and bands in the control group. These findings demonstrate the presence of offline consolidation of a visuo-motor skill, but not of simple motor performance. This effect is predicted by performance on Day 1 and associated with augmented connectivity in the right superior parietal cortex during training.

Disclosures: A.L. Manuel: None. **A.G. Guggisberg:** None. **F. Turri:** None. **A. Schnider:** None.

Poster

557. Motor and Sequence Learning

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Topic: H.02. Human Cognition and Behavior

Support: NIH T32 NICHD 5T32HD055180

Title: Neurobehavioral validation of an individualized behavioral indicator for the presence of incidental explicit awareness in sequential motor learning

Authors: *R. LAWSON, L. A. WHEATON, J. JOHNSON;
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Abstract: Prior studies have demonstrated that the presence of explicit awareness in motor learning is associated with neural changes, impacts consolidation, and can have detrimental effects on motor performance. However, the majority of these studies utilize an intentional paradigm to study the impact of explicit awareness. Due to the challenging nature of identifying when a subject has incidentally developed awareness, it is currently unknown how this method of developing awareness impacts motor learning and subsequent performance. The aim of this study is to provide neurobehavioral validation of a previously identified indicator for the presence of incidentally developed explicit awareness. Twenty, naïve subjects were exposed to a visuomotor task which contained a seven-key repeating sequence unknown to subjects. Subject response latency and accuracy were recorded and compared to the previously established behavioral threshold for prediction of the presence of awareness. EEG measurements were made concurrently to identify network changes occurring during the task. Changes in coherence were utilized as a neural verification of the network shift expected when a subject shifts motor behavior from an externally guided movement to an internally driven execution of the discovered sequence. Establishing a behavioral indicator of such incidentally developed explicit awareness provides a tool in which to further examine the impact of such awareness in motor learning.

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Poster

557. Motor and Sequence Learning

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National Institute of Child Health and Human Development (1R01HD086888-01)

Office of Naval Research (Young Investigator)

Title: Structural correlates of individual differences in motor sequence learning.

Authors: *A. E. KAHN[1,6,2], M. G. MATTAR[3], J. M. VETTEL[6,4,7], N. F. WYMBS[9], S. T. GRAFTON[8], D. S. BASSETT[2,5];
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Abstract: Human skill learning is a complex phenomenon requiring coherent communication across distributed networks of cortical and sub-cortical brain regions [1]. The fine-scale temporal coordination of these regions is critically dependent on white matter tracts that form direct anatomical links, allowing effective transmission of information across the brain [2]. We hypothesized that individual variation in white matter tracts subserving inter-cortical circuits activated by skill learning would be correlated with individual differences in the rate at which subjects learned a set of new motor sequences. Subjects were trained on a Discrete Sequence Production task, wherein they practiced a sequence of key presses following a series of visual cues. Subjects practiced both in-scanner and at home for a total of six weeks. For each subject, diffusion-weighted scans were collected at four time points, one prior to training and then three more at following two-week intervals. Deterministic tractrography was performed separately for each scan to generate connectivity matrices representing white matter streamlines linking cortical and sub-cortical brain regions. Due to their critical role in this particular type of learning [3], we focused our examination on broad swaths of motor and visual regions that form functional modules during task execution. We found that increased white matter connectivity linking early visual regions strongly predicts inter-subject differences in rate of learning: greater connectivity being associated with better learning. Moreover, we found that the strength of multi-edge paths between motor and visual modules was also correlated with learning rate, supporting the role of polysynaptic connections in successful skill acquisition. Our results demonstrate that the combination of diffusion imaging and tractography-based connectivity can provide predictive information about individual differences in learning, particularly when combined with network analysis methods.
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[2] P. Hagmann, L. Cammoun, X. Gigandet, R. Meuli, C. J. Honey, V. J. Wedeen, and O. Sporns, "Mapping the structural core of human cerebral cortex.," PLoS biology, vol. 6, p. e159, July 2008.
[3] D. S. Bassett, M. Yang, N. F. Wymbs, and S. T. Grafton, "Learning-induced autonomy of sensorimotor systems.," Nature Publishing Group, vol. 18, pp. 744-751, May 2015.

Disclosures: **A.E. Kahn:** None. **M.G. Mattar:** None. **J.M. Vettel:** None. **N.F. Wymbs:** None. **S.T. Grafton:** None. **D.S. Bassett:** None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 557.05/MMM26

Topic: H.02. Human Cognition and Behavior

Support: University of Michigan Nicholas Leoni Fund

Title: Neural correlates of focus of attention during golf putting

Authors: *L. SUZUKI, S. K. MEEHAN;
Sch. of Kinesiology, Univ. of Michigan, Ann Arbor, MI

Abstract: Verbal instructions have the potential to influence strategies used during motor skill acquisition and improve learning. For instance, the instructions can be worded to promote either an external focus with emphasis on elements of the environment such as the movement of a golf club or an internal focus of attention, that emphasizes elements of the body such as the position of the hands. The superiority of the external focus of instructions has been demonstrated for a variety of tasks but the brain correlates associated with each type of instructions remain unknown. Therefore we measured brain activity with functional near infrared spectroscopy (fNIRS) while participants practiced golf putting under instructions stressing different foci of attention. Participants were pseudo-randomly assigned to the internal focus or the external focus practice group based upon initial putting performance at a distance of 300 cm from the hole. Participants assigned to the internal focus group were then given three instructions that stressed kinematics of the arm/hand. Participants assigned to the external focus group were given three similar instructions that stressed the resulting movement of the putter. Both groups subsequently performed six practice blocks of thirty putts at varying distances. Golf putting performance was quantified using a modified scoring system that awarded decreasing points for putts that stopped further from the hole. fNIRS was recorded throughout the session using optodes positioned over the bilateral frontal and sensorimotor cortices. Contrary to previous research our preliminary results show that the internal focus group demonstrated greater improvement in golf putting performance from the pre to posttest. The fNIRS results revealed that improved putting performance for the internal group was associated with greater motor but less dorsolateral prefrontal cortex activity during swing execution following practice. In the external focus group the pattern of activation was reversed with more activation in the dorsolateral prefrontal cortex. Dorsolateral prefrontal cortex has been associated with the declarative memory system. The increased dorsolateral prefrontal cortex activity in the external focus group may reflect a greater reliance upon explicit strategies at the expense of skill proceduralization for this task.

Disclosures: **L. Suzuki:** None. **S.K. Meehan:** None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 557.06/MMM27

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI Grant Number 15K21602

JSPS KAKENHI Grant Number 15H01846

Title: Reactivation of declarative memory is essential for the enhancement of the sequential motor skill

Authors: *S. K. SUGAWARA[1,2,3], Y. H. HAMANO[1,4,3], N. SADATO[1,4];
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Abstract: Motor sequential skill is consolidated by sleep. The hippocampus seems to condition the sleep consolidation through interaction with the striatum during training (Albouy et al. 2013). The striatum is related to the chunk formation of the sequence (Penhune and Steele, 2012), whereas the hippocampus is believed to associate temporally discontiguous but structured information during the early phase of motor sequence learning (Albouy et al. 2008; Schendan et al. 2003). It is unknown how the interaction between two structures conditions the sleep consolidation. Considering that the interaction is competitive during the training period (Albouy et al. 2008), we hypothesized that the imbalance between them will affect the sleep consolidation.One hundred five healthy righthanded volunteers participated in this fMRI study with sequential finger tapping learning task for two consecutive days. In reactivation group (n = 58), participants exercised one sequential finger-tapping skill as fast and as accurate as possible (maximum mode) alternating with constant mode with 2 Hz controlled by visual cue, the latter of which requires the retrieval of the learned skill with exact sequential order. In control group (n = 47), participants only performed the maximum mode. 24 hours after the training, all participants retested the learned skill. Considering association of consecutive finger movements during constant mode as reactivation of declarative memory, we expected that its reactivation during training leads to imbalance between hippocampus and striatum, which in turn may preoccupy the skill enhancement.At the end of first day, performance of the reactivation group was significantly greater than that of the control group. Meanwhile, the sleep consolidation was significant in the control group but not in the reactivation group. In addition, at the end of first day, the task-related activity in the declarative memory system including hippocampus and posterior cingulate cortex was significantly greater in the reactivation group compared with the control group. On the other hand, the offline increment of task-related activation was

significantly more prominent in the declarative memory system of the control group than those of the reactivation group. These results show that the activation in the declarative memory system is associated with the skill enhancement during training in the reactivation group, as well as during sleep in the control group. We concluded that the reactivation of declarative memory component of the sequential motor skill is critical for both instantaneous skill enhancement during training and the conditioning for sleep consolidation.

Disclosures: S.K. Sugawara: None. **Y.H. Hamano:** None. **N. Sadato:** None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

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Program#/Poster#: 557.07/MMM28

Topic: H.02. Human Cognition and Behavior

Support: NIH R01 HD084645

Title: Age impacts motor performance but not motor learning in both expected and unexpected events

Authors: *C.-L. YEN, R. K. SHIELDS;
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Abstract: Age may be a moderating factor which influences motor performance and motor learning due to changes in the central nervous system. Despite the fact that motor performance declines in old age, old adults may be able to learn new motor skills by practice, possibly through physiological and behavioral adaptations. Previous studies have shown that aging does not deteriorate the ability to acquire a new motor skill in expected events, such as visual motor and ballistic tasks. However, there have been no studies investigating whether age influences the ability to learn to respond to unexpected events. **Purpose:** The purpose of this study was to determine whether age would influence the ability to learn to respond within the transcortical reflex timeframe following unexpected events at different speeds and resistance levels. We hypothesized that old adults would attenuate the capacity to learn to respond in the transcortical reflex timeframe because older adults show reduced non-dominant cortical excitability. **Methods:** Thirty old (60-80 y/o) and 38 young healthy adults tracked a target set at 3 speeds (Slow-72, Medium-126, Fast-180 degree/second) and 3 resistance levels (Low-10%, Medium-17.5%, High-25% maximal voluntary isometric contraction) using the left wrist before and after motor training over 1 week (Day1Pre, Day1Post, Day3, Day7). Unexpected stretches were imposed to the wrist extensor muscles by releasing the resistance of the device. We calculated

the error to measure learning. Repeated measures ANOVAs were used to compare differences across 4 time points, 2 groups, and conditions with 3 speeds or resistance levels. **Results:** Although old adults showed increased errors in all trials, both old and young adults showed a 2-3 degree reduction in error over both perturbed and unperturbed conditions in the transcortical reflex timeframe from Day1pre to Day7. No difference was shown between Day1post, Day3 and Day7. Moreover, both groups showed the highest errors in the Fast condition. However, practice caused a similar improvement in conditions with each speed. Compared to young adults, old adults demonstrated a similar amount of improvement in all 3 speeds and 3 resistance levels over both perturbed and unperturbed events. **Conclusions:** This study showed clear evidence that despite higher errors in old adults; old adults did not lose the capacity to learn in both expected and unexpected events with all speeds and resistance levels. These findings assist in designing novel rehabilitation interventions for people with aging or neurological diseases.

Disclosures: C. Yen: None. **R.K. Shields:** None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 557.08/MMM29

Topic: H.02. Human Cognition and Behavior

Support: Stress and Motivated Behavior Institute, Syracuse, NY

Title: Acquisition of conditioned avoidance while breathing enhanced CO2 (4%) in humans: sex differences and behavioral inhibition

Authors: *D. P. MILLER[1,2], P. F. MARTINO[1], J. R. MILLER[1], M. T. ALLEN[2,3], J. SHEYNIN[4,5], C. E. MYERS[6,7], R. J. SERVATIUS[2,8];
[1]Neurosci., Carthage Col., Kenosha, WI; [2]Stress and Motivated Behavior Inst., Syracuse, NY; [3]Psychology, Univ. of Northern Colorado, Greeley, CO; [4]Veterans Affairs Ann Arbor Healthcare Syst., Ann Arbor, MI; [5]Dept. of Psychiatry, Univ. of Michigan, Ann Arbor, MI; [6]Pharmacology, Physiol. & Neurosci., Rutgers-New Jersey Med. Sch., Newark, NJ; [7]Dept. of Veteran's Affairs, VA New Jersey Hlth. Care Syst., East Orange, NJ; [8]Rutgers Biomed. Hlth. Sci., Newark, NJ

Abstract: Behaviorally inhibited temperament and female sex have been demonstrated to be vulnerabilities for the development of anxiety disorders. We have suggested that the critical nature of avoidance in diagnosing anxiety requires the study of avoidance acquisition and vulnerabilities, which has been effectively examined in non-humans (e.g., Servatius et al, *Beh Brain Res*, 192, 2008). In humans, Sheynin et al (e.g., *Frn Beh Neurosci*, 8:323, 2014) utilized a

computer-based task to demonstrate sex and temperament differences in avoidance acquisition. We used this task to examined avoidance acquisition while participants breathed enhanced CO2 (4%), which activates stress response. We hypothesized that activating stress response would alter learning and physiological responding in vulnerable individuals. Participants were undergraduates between the ages of 18-22. Behavioral inhibition characteristics were categorized based on responses on the Adult Measure of Behavioral Inhibition. Participants were fitted with a Hans Rudolph face mask apparatus and allowed to breath air for 15 minutes to allow heart rate to return to baseline levels. Avoidance acquisition was accomplished using a computer-based spaceship video game (courtesy of Sheynin and Myers). During avoidance acquisition, all participants inhaled 4% CO2 enhanced air during the first 12 trials of training. The following 12 trials were extinction trials, during which all participants inhaled air through the face mask system. Overall, we demonstrated that individuals successfully acquired conditioned avoidance in the computer-based spaceship video game while inhaling 4% CO2 enhanced air. Females appeared to show some deficits in extinction compared to males. Further, males made more moves, more shots, and scored more points than females. Behaviorally inhibited individuals and females showed decreased locomotion. Our results appear to be in agreement with previous research using the spaceship video game where participants directly inhaled normal air (e.g., Sheynin et al., 2014). Our procedure is effective in examining cognitive processes while inducing stress in participants.

Disclosures: **D.P. Miller:** None. **P.F. Martino:** None. **J.R. Miller:** None. **M.T. Allen:** None. **J. Sheynin:** None. **C.E. Myers:** None. **R.J. Servatius:** None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

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Program#/Poster#: 557.09/MMM30

Topic: H.02. Human Cognition and Behavior

Support: Departmental funding (Department of Psychiatry, University of Michigan)

Title: The cognitive basis of human avoidance behavior

Authors: *J. SHEYNIN[1,2], S. BAIDYA[3], I. LIBERZON[1,2];
[1]Veterans Affairs Ann Arbor Healthcare Syst., Ann Arbor, MI; [2]Dept. of Psychiatry, [3]Col. of Literature, Science, and the Arts, Univ. of Michigan, Ann Arbor, MI

Abstract: While avoidance behavior can be an adaptive behavior that protects one from harm, excessive avoidance might be maladaptive and be linked to the development of

psychopathology. Indeed, excessive avoidance behavior is a key symptom in all anxiety disorders and posttraumatic stress disorder (PTSD). In both human and non-human animal research, avoidance is often studied in the context of fear conditioning, where the subject is required to perform a specific response (e.g., pressing a keyboard/lever) to prevent an aversive event (e.g., electric shock). On such paradigms, it has been a challenge to dissociate the two commonly theorized drivers of avoidance behavior, namely, fear and cognitive expectancy. Here, we used a recently developed computer-based task that uses a mild aversive event, to focus on the cognitive aspect of avoidance behavior. On this task, the participant controls a spaceship avatar and seeks to get a high score (later translated into monetary award) by shooting an "enemy" spaceship that moves on the screen. During the task, different "warning stimuli" that predict an aversive event (points loss) in some cases, appear on the screen. The participant learns that hiding the spaceship in designated "safe areas" protects from the aversive event but also prevents the opportunity to gain reward (points). Such avoidance response was studied in three distinct phases: (1) acquisition, where participants learned the avoidance response, (2) generalization, where participants demonstrated avoidance response to stimuli that share some similarities with the previously conditioned warning stimuli, and (3) operant extinction, where stimuli are not associated with the aversive events anymore, and participants gradually decreased their avoidance responding. Results show that healthy adults successfully learned the avoidance response on this task, validating this task for studies of learned avoidance. Interestingly, participants with anxiety vulnerabilities demonstrated greater avoidance responding, suggesting that excessive avoidance might put one at a higher risk to develop psychopathologies. Lastly, self-reported expectancy of the aversive events was associated with the degree of the avoidance behavior. Implication of these findings to psychotherapy, as well as future follow-up directions will be discussed.

Disclosures: **J. Sheynin:** None. **S. Baidya:** None. **I. Liberzon:** None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

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Program#/Poster#: 557.10/MMM31

Topic: H.02. Human Cognition and Behavior

Support: University of Northern Colorado

Stress and Motivated Behavior Institute

Title: Exercise effects on classical eyeblink conditioning in humans

Authors: *T. ALLEN;
Sch. Psych Sci., Univ. Northern Colorado, Greeley, CO

Abstract: The benefits of exercise for neural function are well known. A study in male rats has found that exercise improves eyeblink conditioning (Green et al., 2011). There were no exercise effects on pseudo-conditioning with unpaired presentations of the tone CS and the air puff US. The current study was conducted to determine if exercise enhances eyeblink conditioning in humans. In this preliminary study, we used self-report of normal exercise habits to group our participants as high or low exercise. We hypothesized that participants self-reporting higher levels of weekly exercise would exhibit enhanced acquisition of conditioned eyeblink, but that there would be no differences in response to unpaired CS and US trials. Sixty seven undergraduates (14 males and 53 females, mean age = 19.6 years) voluntarily participated for research credit. All participants completed a questionnaire about their exercise habits. Participants were grouped as low exercise (one hour or less per week) or high exercise (3 or more hours per week). Thirty participants in the acquisition condition received 6 blocks of ten trials each of which consisted of 8 paired trials with a 500 ms tone CS which overlapped and co-terminated with a 50 ms, 5 psi corneal air puff US along with a US alone trial and a CS alone trial. Thirty seven participants in the pseudo-conditioning control condition received 60 pseudo-randomly presented CS alone and US alone trials. Pseudo-conditioning resulted in no significant increase in eyeblinks to the tone. There was a decrease in UR amplitude across US alone trials in the pseudo-conditioning in both high and low exercise individuals. Low exercise participants exhibited enhanced CR acquisition as compared to high exercise participants. There were no differences between high and low exercise groups in UR amplitude on US alone test trials across six blocks of acquisition training. Surprisingly, we found the opposite effects for exercise in human eyeblink conditioning as was previously reported in male rats. Methodological differences between the prior rat study and the current human study including gender and eyeblink evoking stimuli may account for inconsistent results across species. Possible neural mechanisms in the hippocampus and cerebellum as well as stress and anxiety effects involving exercise and eyeblink conditioning will be discussed.

Disclosures: T. Allen: None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 557.11/MMM32

Topic: H.02. Human Cognition and Behavior

Support: FARB grant from University of Naples, ORSA140458

Title: Method to evaluate human learning rate of movement control

Authors: *A. VIGGIANO[1], G. RAGOGNETTI[2], L. LORUSSO[1], A. MARCELLI[2];
[1]Med. and Surgergy, Univ. of Salerno, Baronissi, Italy; [2]Dept. of Information and Electrical Engin. and Applied Mathematics, Univ. of Salerno, Fisciano, Italy

Abstract: One intriguing approach to understand the mechanism for human learning is to formulate a mathematical model of the operant conditioning. The learning principle of such models is the update of the synaptic weights of some of the neurons of the network when a reinforcement signal occurs. The rule for such update requires establishing the amount of changes in the synaptic weights. This consists often of a parameter "α"chosen in such a way to obtain a "good" performance of the model. The aim of the present research was to estimate a possible "physiological" value for the parameter α in humans. To this end, a group of volunteers were asked to learn to control the movement of a robotic head to point it toward a light source through a computer keyboard. The participants received a graphical feedback on the monitor correlated to the light intensity and the position of the robotic head and a success or failure signal following the movements that they chose. The feedback consisted of three vertical bars representing respectively the light intensity at two photosensors placed on the two sides of the robot head (first and second bar) and the position of the head (third bar); each bar had three possible levels. Looking at the graphical feedback, the participants had to chose to press one out of three keys; each key moved the robotic head on one out of three positions (center, left, right). After the movement was chosen, a positive signal (a green frame and a bell sound) was given if the head pointed toward the light source; otherwise a negative signal was given (a red frame and a buzz sound). The position of the light source changed randomly after the positive (or negative) feedback was given. The participants, however, did not see the robotic head and were blind about the real meaning of the information appearing on the screen and the movements consequent to the key press. The results demonstrated that peoples correctly learned to point the robotic head toward the light and that their learning curve was compatible with an α value smaller than 0.006; a correlation has been observed between the learning rate and the variability of subjects' responses. Furthermore, learning curves obtained with different levels of complexity of the task were also evaluated. The results support a theoretical exponential relationship between the α value and the inverse of the dimension of the training ensemble of stimuli. Finally, the variability of the experimental data between subjects was smaller for more complex tasks, suggesting that other personal factors, like previous experiences, contribute with a greater extent in simpler tasks; this factors should be taken into account to define the "simplicity" of a task.

Disclosures: **A. Viggiano:** None. **G. Ragognetti:** None. **L. Lorusso:** None. **A. Marcelli:** None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: KTIA NAP 13-2-2015-0002

Janos Bolyai Research Fellowship

Title: Causal evidence of the involvement of the DLPFC in implicit statistical learning

Authors: *D. NEMETH[1,2], G. G. AMBRUS[3], K. JANACSEK[2], A. TRIMBORN[3], G. KOVACS[3];
[1]Inst. of Psychology, Eotvos Lorand Univ., Budapest, Hungary; [2]Hungarian Acad. of Sci., Budapest, Hungary; [3]Friedrich Schiller Univ., Jena, Germany

Abstract: Human learning depends on multiple cognitive systems related to dissociable brain structures. These systems interact not only in cooperative but sometimes competitive ways in optimizing performance. Previous studies showed that manipulations reducing the engagement of frontal lobe-mediated explicit, attentional processes can lead to improved performance in striatum-related procedural learning. The aim of the present study was to investigate the role of the prefrontal cortex (PFC) in implicit statistical learning and its consolidation and to explore the competitive relationship between implicit statistical learning and frontal lobe functions. Healthy, young adults (n=20) were trained on a probabilistic sequence learning task. 1 Hz transcranial magnetic stimulation (TMS) or sham stimulation of both the left and right dorsolateral PFC (DLPFC) was applied right before the learning session and 4x during the learning session in order to disrupt frontal lobe functions. To assess the lasting effects of TMS on learning and consolidation, statistical learning performance (expressed as the faster reaction times for sequences with high as compared to low probabilities) was tested ten minutes, two hours, and 24 hours later. We found significantly lower level of learning in the bilateral DLPFC as compared to the sham stimulation group ten minutes after learning. However the DLPFC stimulation group showed better performance compared to the sham group after the 24-hour consolidation period. Our results support a dynamic antagonist relationship between the brain networks of automatic and controlled processes.

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Poster

557. Motor and Sequence Learning

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Topic: H.02. Human Cognition and Behavior

Support: NIH Intramural Program

CNRM

Title: Robustness of functional and structural brain plasticity in motor sequence learning

Authors: *C. THOMAS[1], A. STEEL[1], A. TREFLER[1], E. AGUILA[1], N. SADEGHI[2], C. PIERPAOLI[2], C. I. BAKER[1];
[1]Lab. of Brain and Cognition, NIMH, Bethesda, MD; [2]NICHD, Bethesda, MD

Abstract: Previous MRI studies have reported changes in human brain function and structure over short-term (hours) and long-term (weeks) training using different tasks. However, given the limited resolution of MRI compared with the likely substrate, and the potential for confounding factors, the robustness of these prior results is unclear. Here, in a group of 21 healthy adults, we employed a carefully controlled lateralized motor sequence-learning paradigm and collected resting-state fMRI (rsFMRI) and structural MRI (sMRI) data to investigate the topography and time-course of training-dependent functional and structural changes in the brain. The task involved learning a specific sequence of eight speeded button presses. Each participant was scanned before and after short-term (90 minutes) or long-term (1 hour/day for one week) training. To test for the specificity of changes to motor learning, active (i.e. videogame training) and passive control scans (i.e. rest) were conducted, and to test the reproducibility of any apparent changes, multiple scans were collected in each session. Finally, to relate the changes to behavior, we tested whether the functional/structural changes correlate with improvements in task performance. Analyses of the rsFMRI and sMRI data revealed significant diurnal changes in both functional and structural metrics such as global connectedness and cortical thickness, respectively. After controlling for these diurnal changes, there was still evidence for changes in some structural and functional measures for training compared to control conditions. However, these effects were widespread, and not always consistent between control scans or replication scans. Additionally, areas of functional and structural change were not limited to the expected motor areas and were found in both hemispheres despite lateralization of the training. We conclude that compared to the effect size of confounds like diurnal changes, the functional and structural changes induced by training do not appear robust.

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Poster

557. Motor and Sequence Learning

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Topic: H.02. Human Cognition and Behavior

Support: NSF 1358756

Title: Practice promotes skill through automatization: evidence from an arbitrary visuomotor association task

Authors: A. D. FORRENCE, R. M. HARDWICK, J. W. KRAKAUER, *A. M. HAITH; Neurol., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: The ability to respond accurately at short latency is a critical component of motor skill (Hardwick et al. 2015; SfN 2015, 806.29). Such rapid responding must typically be acquired through practice. Many skills depend on learning arbitrary associations between stimuli and actions. For example, musical sight-reading requires timely execution of a movement in response to a note on a page—an arbitrary symbolic stimulus. Here we consider whether such short-latency response skills might depend upon automatization through practice.

We tested this hypothesis using an arbitrary visuomotor association task in which four unfamiliar symbols each corresponded to pressing a specific key on a keyboard. Participants responded to a succession of symbol presentations, in a random order, as rapidly as possible (Serial Reaction Time condition; SRT). Feedback after each trial informed participants whether they had pressed the correct key and, if not, which key should have been pressed, enabling to learn the required mapping very rapidly. Participants then practiced this task for multiple days, aiming to improve their time to complete a set number of trials.

To more precisely measure participants' level of skill in this task, we employed a timed response (TR) paradigm to assess their ability to respond at a range of preparation times and thereby infer their speed-accuracy tradeoff. We measured subjects' level of skill this way at the outset of learning and following practice, and found that practice led to substantial improvements in the speed-accuracy tradeoff.

Following practice, we assessed the automaticity of the association between an arbitrary stimulus and its response by transposing the association between two stimulus-finger pairs. Participants practiced this revised mapping under SRT conditions, then performed the TR task so that we could evaluate their ability to express this mapping given varying amounts of time to prepare their response. Performance under the revised mapping in the SRT condition was comparable to that on the first day under the original mapping. However, in the TR condition, although participants could correctly express the revised mapping when allowed long preparation times, they reverted to the originally practiced map when forced to move at short preparation times.

Thus, stressing the reaction time revealed an automatic mode of responding that persisted despite participants successfully learning to comply with the revised requirements of the task. We suggest that a critical component of motor skill is the presence of an automatic mode of response that operates at short latencies.

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Poster

557. Motor and Sequence Learning

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Program#/Poster#: 557.15/MMM36

Topic: H.02. Human Cognition and Behavior

Title: Motor cortical substrates of declarative influence over the procedural system: A short-latency afferent inhibition study

Authors: *S. K. MEEHAN[1], L. Y. SUZUKI[1], J. L. MIRDAMADI[2];
[1]Sch. of Kinesiology, Univ. of Michigan, Ann Arbor, MI; [2]Kinesiology, Indiana University-Bloomington, Bloomington, IN

Abstract: The declarative and procedural memory systems contribute unique elements to motor performance. The different contributions of each system can place them in competition such that the declarative system may impose constraints on the procedural system to match an explicit strategy. We have used short-latency afferent inhibition (SAI) to show that attention demands selectively influence neuronal populations in motor cortex. Activity in the same neuronal population has recently been linked to cerebellar mediated model-based learning. Therefore, we sought to assess the interaction between attention demands and cerebellar excitability in this particular population of neurons using SAI. SAI was elicited with electrical median nerve stimulation at the right wrist that preceded a single monophasic transcranial magnetic stimulus over the left motor cortical representation of the first dorsal interosseous. Motor evoked potentials were elicited using either posterior-anterior (PA) or anterior-to-posterior (AP) monophasic magnetic stimuli. PA magnetic stimuli predominantly recruit early indirect waves while AP magnetic stimulation predominantly recruit a distinct set of oligosynaptic pathways mediating later indirect waves. To assess the effect of attention load the magnetic stimuli for both current directions were delivered while participants engaged in a high or low load visual detection task. To investigate the interaction between attention load and cerebellar excitability participants completed the same combinations of current direction and attention load before and after intermittent theta burst stimulation (iTBS) over the posterior lobe of the cerebellum.

Preliminary results are consistent with our previous work. Attention load had no effect upon SAI elicited using the PA stimulation prior to cerebellar iTBS. Conversely, SAI was reduced under high compared to low attention load for AP stimulation prior to cerebellar iTBS. Additionally, cerebellar iTBS had no effect upon SAI elicited using PA stimulation. Excitation of the cerebellum did reduce the magnitude of SAI elicited using AP stimulation under low attention loads. However, the effect of cerebellar stimulation upon SAI elicited using AP stimulation was attenuated under high attention loads. The results of the current study suggest that attention and cerebellar influences converge on the neuronal populations mediating late indirect waves. The convergence of attention and cerebellar influences on this neuronal population may represent a substrate by which the declarative system shapes motor performance through the control of sensory input to motor cortex.

Disclosures: S.K. Meehan: None. **L.Y. Suzuki:** None. **J.L. Mirdamadi:** None.

Poster

557. Motor and Sequence Learning

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Program#/Poster#: 557.16/MMM37

Topic: H.02. Human Cognition and Behavior

Support: GO-8 Re-LOAD

Title: Effects of a bout of acute exercise on motor learning and EEG frequency band power in older adults

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Abstract: In young adults, an acute bout of high intensity physical exercise, as compared to a resting condition, led to more improvement in motor performance 24 h after exercise, indicating that acute exercise boost motor learning (Roig et al., 2012). On a neurophysiological level studies revealed that EEG alpha (8-12 Hz) and beta (13-30 Hz) power were increased after short bouts of exercise, probably reflecting an enhanced state of physical relaxation or arousal (for a review see Crabbe & Dishman, 2004). However, so far no study used EEG correlates to investigated whether and how acute exercise also benefit motor learning. Further, cardiovascular fitness has been shown to be associated with motor performance (Hübner et al., under review). We aimed to investigate whether acute exercise also affects motor learning and EEG power during motor performance in high-fit older adults. Thirty high-fit older adults (65-74 years of age) are assigned to an experimental group (n = 15, acute exercise: 20 min moderate cycling at

65 % of peak Watt level performed during spiroergometry) and a control group (n = 15, resting condition: listening to an audio book). Groups are matched with respect to their cardiovascular fitness level (assessed with spiroergometry on a bicycle ergometer). Motor learning is assessed with a visuomotor precision grip tracking task. Participants have to track a sine wave pattern with their right dominant hand at baseline (immediately before acute exercise: 8 trials of 15 sec (i.e., 1 block)) and for practice (immediately, 30 min and 24 h after exercise: 4 blocks each). Tracking variability is operationalized as the root mean square error (RMSE) at baseline and of the last practice block. The EEG was recorded during baseline test, immediately and 30 min after practice. Alpha and beta task-related power (TRPow) are analyzed for central and frontal electrodes. Data collection and analysis are still in progress. We expect that motor performance of the experimental group is better 24 h after intervention as compared to the control group, indicating improved motor consolidation processes. With respect to the EEG we expect the experimental group to reveal stronger alpha and beta TRPow decreases immediately and 30 min after exercise in central and frontal electrodes, reflecting enhanced recruiting of cortical resources during motor learning. Crabbe, JB & Dishman, RK (2004). *Psychophysiol, 41*(4), 563-574. Hübner, L, Godde B & Voelcker-Rehage (under review). Roig, M, Skriver, K, Lundbye-Jensen, J, Kiens, B & Nielsen, JB (2012). *PloS One, 7*(9), e44594.

Disclosures: L. Huebner: None. **B. Godde:** None. **C. Voelcker-Rehage:** None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 557.17/MMM38

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI Grant Number 15H01846

JSPS KAKENHI Grant Number 15K21602

Title: Dynamic change of the parieto-premotor network during early motor sequence learning: an fMRI study

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Abstract: Motor sequence learning is known to be mediated by primary motor cortex, putamen, and cerebellum (Dayan & Cohen, 2011; Penhune & Steele, 2012; Loshe et al., 2014). Less evident is the learning related neural network change, particularly during early phase of the learning (~ 1 hour; Penhune & Steel, 2012). Here, we investigate how the neural network alters with early motor sequence learning by using eigenvector centrality mapping (ECM; Lohmann et al., 2010). Regarding each brain voxel as node and their functional connectivity as edge, ECM attributes a centrality value to each voxel such that the voxel receives a large value if it is more strongly correlated with other voxels which are central within the network. Thus ECM maps the importance of each voxel within the whole brain network with seed- and task-free fashion (Lohmann et al., 2010). A total of 58 normal volunteers participated in the functional MRI study with sequential finger tapping task by their non-dominant left hand. We adopted the block design which includes task epoch of constant speed mode with 2 Hz controlled by visual cue alternating with rest epoch. We first modeled out the task-related activity to extract residuals. Then, we sorted the residual time series data of each task and rest epochs separately, and concatenated these data to estimate whole-brain ECM. By evaluating the increase of centrality during task-state and during resting state separately, we observe learning related network changes which is state-dependent. As learning progressed, the centrality during task state was significantly increased in bilateral posterior parietal cortex. As the intraparietal sulcus regions are critical in visuo-motor integration, and as the task is the visually cued sequential finger tapping, the enhanced centrality in the posterior parietal cortex may represent the learning of cue-tap synchronization. Meanwhile, the centrality during rest-state was significantly increased in bilateral dorsal premotor areas and anterior parietal cortex. As no task was performed, these areas are supposed to be critical nodes for the network representing the spatial and temporal features of the skill *per se.* Our present findings are consistent with the notion that the parieto-premotor network codes the learned sequential finger tapping skill (Kornysheva & Diedrichsen, 2014). The anterior network holds the memory of the sequence *per se*, whereas the posterior network is related to visuo-motor coordination. We conclude that, in addition to the M1, cerebellum, and the striatum, the cortical network including premotor area and parietal cortex holds the memory of the learned sequential skill even in the early learning stage.

Disclosures: Y.H. Hamano: None. **S.K. Sugawara:** None. **N. Sadato:** None.

Poster

557. Motor and Sequence Learning

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NIMH Grant 5T32MH019118-23

Title: Investigating motor adaptation in essential tremor and the influence of VIM thalamic deep brain stimulation

Authors: *J. A. GUERIN[1,2], S. LEE[1,2], M. AHN[1,2], S. R. JONES[1,2], W. F. ASAAD[1,2,3,4,5]; [1]Neurosci., [2]Brown Inst. for Brain Sci., [3]Neurosurgery, Alpert Med. Sch., Brown Univ., Providence, RI; [4]Neurosurg., [5]Norman Prince Neurosciences Inst., Rhode Island Hosp., Providence, RI

Abstract: Essential Tremor (ET), the most common adult movement disorder, has been historically considered a monosymptomatic disorder and is characterized by a debilitating, involuntary action tremor (4–12 Hz). ET is thought to arise from abnormal cerebellar function, and some evidence suggests that ET patients may have additional motor deficits. For medication-refractory ET, deep brain stimulation (DBS) of the cerebellar thalamus, the ventral intermediate nucleus (VIM), can be an effective treatment. While DBS is principally effective in reducing tremor magnitude, further investigation into its effects on non-tremor symptoms is needed. We sought to investigate (1) whether patients with ET exhibit motor learning deficits and (2) the extent to which DBS influences motor learning.

ET patients and age-matched controls performed a modified center-out joystick task in which they moved a cursor on screen to one of eight randomly appearing targets. During blocks of standard trials, joystick and cursor trajectories were mapped naturally. In some blocks of trials, the mapping between the joystick movement and cursor trajectory was rotated counterclockwise by 45 degrees, requiring an adjustment in movement to successfully get the cursor to the target. In other blocks, cursor trajectories were rotated randomly across trials either by 45 degrees counterclockwise or by 45 degrees clockwise. Intermittent probe trials were also included in which the cursor movement was mapped to the ideal trajectory irrespective of the subject's real joystick movement, thus providing no visual feedback about movement error.

To assess motor learning, we investigated motor adaptation, deadaptation, and savings. We measured path length on each trial by isolating gross movement from tremor frequency activity by low-pass filtering cursor trajectories and calculating the cumulative vector distance between consecutive joystick points. Path lengths were compared between (1) non-DBS ET patients and age-matched controls and (2) DBS 'ON' and DBS 'OFF' states in postoperative ET patients. Our preliminary data suggest that ET patients demonstrate deficits in adaptation relative to healthy controls and that deficits may be exacerbated in DBS 'ON' states. In addition, ET patients undergoing awake surgery for DBS electrode implantation performed this task while we simultaneously recorded spikes and local field potentials from VIM and surface potentials from sensorimotor cortex using electrocorticography. Together, these data may provide further evidence of additional motor symptoms in ET and potential deficits introduced with DBS.

Disclosures: **J.A. Guerin:** None. **S. Lee:** None. **M. Ahn:** None. **S.R. Jones:** None. **W.F. Asaad:** None.

Poster

557. Motor and Sequence Learning

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant NS44393

Institute for Collaborative Biotechnologies through grant W911NF-09-0001 from the U.S. Army Research Office.

Title: Longitudinal representational similarity analysis of extensive motor sequence learning

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[1]Psychological & Brain Sci., [2]Sage Ctr. for the Study of Mind, UCSB, Santa Barbara, CA;
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Abstract: Introduction: We previously used repetition suppression functional magnetic resonance imaging (fMRI) to show that representations for newly acquired motor sequences appear over time-scales that vary significantly from region to region. Here, we extend these results by using representational similarity analysis (RSA) on the same longitudinally acquired fMRI data to identify regions where neural representations of sequence specific movements are consistently strengthened over 6 weeks. We hypothesized that multivariate sequence representations in several key motor regions including M1 and premotor cortex will show evolving representational patterns reflecting the depth of training. Specifically, we predicted that long-term, training would lead to increasing multivoxel dissimilarity measures for two extensively trained sequences as well as increasing dissimilarity with other sequences that were not trained extensively.
Methods: 20 healthy subjects practiced 6 motor sequences daily, over 6 weeks at different levels of exposure (extensive, moderate, minimum). Each sequence consisted of 10 button presses performed with the right hand (2 presses per finger). Subjects also underwent a total of 4 fMRI sessions, one before training and then, every two weeks during training. In every fMRI session each motor sequence was performed 50 times resulting in a total of 300 trials. BOLD data recorded during sequence performance in an event-related design was analyzed in the framework of a GLM and resulting beta images were used to calculate dissimilarity matrices in a set of regions of the motor system.

Results: Dissimilarity matrices were computed comparing the relation of neural activation elicited by the sequences trained at different intensities, derived from the 4 fMRI-sessions. Dissimilarity patterns changed between sessions for several motor regions. Increasing dissimilarity was observed between the two extensively trained sequences with each other as well as when they were compared with the minimally trained sequences in the left PMv, SMA, right PMd and parietal cortical areas.
Conclusions: We observed a distinct longitudinal pattern of change in multivariate sequence representations over time, indicating the regionally represented informational content associated with a specific sequence evolves systematically different over time. Our findings indicate the formation of highly sequence-specific representations in PMv and SMA, which still evolve after extensive training and may therefore drive sequence learning on an ultra-slow timescale as proposed by recent multi-process models of motor learning.

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Poster

557. Motor and Sequence Learning

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Topic: H.02. Human Cognition and Behavior

Support: NIH R01 HD082109

Title: Cognitive-motor dual-task training influences consolidation and transfer of learning during a novel visuomotor weight-bearing task

Authors: *K. R. COLE, R. K. SHIELDS;
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Abstract: Dual-task training, or training a motor task while performing a simultaneous cognitive task, may improve automaticity of movement. Dual-task training may be essential for functional mobility (e.g. walking and talking). **Purpose**: We determined the effects of dual-task training on motor skill acquisition and retention of a visuomotor weight-bearing task. We also determined the generalizability of this learning to a new task. **Methods**: Forty healthy adults (18-36 years old) performed a visuomotor task, tracking a sinusoid on a screen while performing a single limb squat. Every 1.0s, an upright or inverted ‘T’ of four different colors flashed on the screen for 0.5s. Two control groups (20 subjects) performed solely the motor task, while ten were instructed to also sum one ‘T’ color/orientation (DT1), and another ten subjects to sum two ‘T’ color-orientations (DT2). Day1 consisted of 20 training trials at a medium speed and resistance.

Subsequently, each performed the motor task under a 3x3 matrix of resistances as percent of body weight (5, 10, 15%) and velocity (.2, .4, .6 Hz) of the target. Two days later, subjects performed five training trials under their original condition, followed by the 9 novel conditions of the cross-condition. One-way ANOVA (significance of 0.05) was used to determine differences between groups for error (target - user position). **Results**: All groups demonstrated a significant learning effect after training ($p=0.05$); albeit the number of trials to achieve proficiency was greater in the DT2 group compared to controls and DT1 ($p=0.014$). Limited carryover of the learned behavior was present for DT during slow velocity ($p<.0001$), while no difference in error was evident at the fast velocity, supporting carryover. 48hrs later, all groups performed similarly on the first trial ($p=0.45$), though after 5 trials, those in the control group achieved 25% less error compared to the DT2 group ($p=0.01$). **Conclusion**: Young adults performing dual-task training require additional training to achieve a similar proficiency when compared to training without the dual-task strategy. Dual-tasking does not influence motor task consolidation; however, it does continue to affect further training. Interestingly, performing a difficult cognitive task allows transfer of motor skill to faster speeds, though not slower speeds. This may be due to increased reliance on automaticity (decreased attention to the motor task) during faster conditions, rather than attending to both cognitive and motor tasks at slower speeds. Future work will examine dual-task training effects on those with movement impairments due to age and neuromuscular compromise.

Disclosures: **K.R. Cole:** None. **R.K. Shields:** None.

Poster

557. Motor and Sequence Learning

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Topic: H.02. Human Cognition and Behavior

Support: South China Normal University

Chiang Jiang Scholars Program, Ministry of Education, China

Guangdong Foreign Experts Recruitment Program

National Natural Science Foundation, China, 31371050

Title: Effector specificity in procedural category learning

Authors: K. JENTINK[1], A. I. WILSON[1], H. MECHTENBERG[1], Z. LIU[2], *C. A. SEGER[1,2]; [1]Psychology, Colorado State Univ., Fort Collins, CO; [2]South China Normal Univ., Guangzhou, China

Abstract: There is debate about the role of motor systems in category learning. One theory is that subjects learn direct mappings from stimuli to motor responses, and therefore learning should be specific to particular motor effectors. This theory is supported by studies finding that disrupting the stimulus - motor response relationship by altering the motor response can interfere with category learning. Alternatively, subjects may learn an intermediate category representation, learning to map each stimulus to a category label, and separately learning to map the category label to a motor response. If an intermediate category representation is learned, it should be accessible to any motor effector and disruption of the category-response mapping should not affect categorization. Our study sought to identify the neural systems recruited during category learning with consistent versus inconsistent category-response mappings using functional magnetic resonance imaging (fMRI). Thirty participants learned an information-integration category task outside the scanner, half with consistent mappings, and half with inconsistent mappings that changed on a trial by trial basis. All subjects were then scanned while performing the task under the same conditions. Participants in both consistent and inconsistent mapping conditions had similar accuracy during scanning, indicating that all were able to learn. A direct comparison showed a striking overlap between consistent and inconsistent mapping groups: both conditions recruited regions frequently associated with category learning, including the basal ganglia, intraparietal sulcus, and premotor and motor cortexes. A direct comparison found greater activity for the inconsistent mapping in the intraparietal sulcus, medial occipital regions, and globus pallidus. Our results support theories of category learning that include an intermediate category representation, rather than learning being limited to specific stimulus-motor response mappings. Both behavior and neural systems were very similar across conditions. Our results further point to a role of the intraparietal sulcus and medial occipital regions in determining and implementing the current category - motor response mapping.

Disclosures: K. Jentink: None. **A.I. Wilson:** None. **H. Mechtenberg:** None. **Z. Liu:** None. **C.A. Seger:** None.

Poster

557. Motor and Sequence Learning

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Topic: H.02. Human Cognition and Behavior

Support: KTIA-NAP 13-2-2015-0002

Janos Bolyai Research Fellowship

Title: Age-related differences in the consolidation of implicit statistical memory across human life span: Evidence from a probabilistic sequence learning task

Authors: *K. JANACSEK[1,3], D. JUHÁSZ[4], D. NEMETH[3,2];
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Abstract: Statistical learning is a fundamental mechanism of the brain which extracts and represents regularities of our environment. It underlies predictive behavior and decision making in many day-to-day activities. Therefore it is crucial to understand how we form associations based on statistical regularities, and how the acquired information consolidates and stabilizes over time. The ontogenetic changes of these processes, however, are still poorly understood. Here we aimed to characterize age-related differences in the consolidation of statistical memories between 7 and 85 years of age. Three-hundred participants were recruited and clustered into nine age groups. The Alternating Serial Reaction Time (ASRT) task was used to assess implicit statistical learning. Participants were retested 24 hours after the learning phase. Although all groups showed retention of the acquired statistical regularities after the 24-hour consolidation period, we found greater inter-individual variability in the extent of retention in childhood between 7 and 13 years of age and in the elderly population (60-85 years of age). These results remained stable even after controlling for age-related differences in overall accuracy and reaction time. Our findings suggest that the fronto-striatal circuits mediating statistical memory formation and consolidation undergo marked changes in childhood and in late adulthood, while seem to be well-established in adolescence and adulthood.

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Poster

557. Motor and Sequence Learning

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Program#/Poster#: 557.23/MMM44

Topic: H.02. Human Cognition and Behavior

Title: The impact of reward and punishment on the neural correlates of sequence learning

Authors: *A. D. STEEL[1,2], E. H. SILSON[1], C. J. STAGG[2], C. I. BAKER[1];
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Abstract: While several studies have examined the impact of feedback on skill learning, the multitude of different approaches and paradigms used make synthesizing general principles problematic. Here, we employed closely matched paradigms across two different tasks to assess the impact of feedback to sequence learning and retention, both behaviorally and neurally. Participants (n=72) performed either the serial reaction time task (SRTT, n=36) or force tracking task (FTT, n=36) with positive-, negative-, or pseudo-feedback during fMRI, with 20 mins resting-state data collected pre- and post-learning. In the SRTT, participants responded to a stimulus presented in 1 of 4 locations by pressing a corresponding button. In the FTT, participants modulated their grip force to move a cursor on the screen and track a moving target. In both tasks, unbeknownst to the subject, the stimulus followed a fixed sequence during certain blocks of trials. The difference in performance between fixed and random blocks indexed learning. Participants received feedback based on their performance. For the SRT, feedback was based on speed and accuracy; for FTT, feedback was based on distance from the target. The reward (REW) group was given monetary reward if subjects improved compared to the previous block. The punishment (PUN) group had money taken away if they were worse than the previous block. Control (CON) participants were told simply that they would be given compensation based on their performance. Behaviorally, PUN aided performance during SRTT, but impaired performance during FTT. Feedback did not alter retention at any time point. In the resting-state data, task-responsive voxels were clustered into networks based on their functional connectivity (FC). The FC of these networks was compared pre- and post- training. Different FC profiles predicted retention at 1-hour and at 24-hours post-learning: increased post-learning FC between basal ganglia (BG) and motor cortex was predicted by retention after 1-hour, while 24-hour retention was correlated with the average BG FC to all networks after learning. Neither task nor feedback significantly influenced these relationships. In sum, short-term retention is related to specific cortical-BG connections, whereas intermediate- and long-term retention is related wide-spread integration via BG. These results support the hypothesis that the BG play a critical role in memory formation independent of feedback given during learning. Together with our behavioral result, these findings suggest that the effects of feedback are transient and call into question the previous findings that reward aids the retention of motor skills

Disclosures: **A.D. Steel:** None. **E.H. Silson:** None. **C.J. Stagg:** None. **C.I. Baker:** None.

Poster

557. Motor and Sequence Learning

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Research and Development funding from the NHS Executive

Title: Somatotopic cerebellar abnormalities in developmental amnesia due to bilateral hippocampal damage and in verbal/orofacial dyspraxia associated with bilateral caudate atrophy.

Authors: *G. P. ARGYROPOULOS[1], K. S. SALEEM[2], M. MISHKIN[2], F. VARGHA-KHADEM[1,3];
[1]Cognitive Neurosci. and Neuropsychiatry Section, UCL Inst. of Child Hlth., London, United Kingdom; [2]Lab. of Neuropsychology, Natl. Inst. of Mental Health, Natl. Inst. of Hlth., Bethesda, MD; [3]Great Ormond Street Hosp. for Children NHS Fndn. Trust, London, United Kingdom

Abstract: Introduction
We previously described two distinct neurodevelopmental disorders, one labelled 'developmental amnesia' (DA), resulting from hippocampal atrophy due to neonatal hypoxia-ischaemia[1], and the other, a 'verbal and orofacial dyspraxia' (VOD), associated with atrophy of the caudate nucleus due to a point mutation in the gene *FOXP2*[2,3]. As both the hippocampus and the caudate nucleus connect with the cerebellum[4,5], we sought to determine the precise location of cerebellar structural abnormalities in these two disorders.
Methods
We used cerebellar VBM to compare gray matter volumes in the MRIs of 8 DA patients and 7 VOD cases with those of 13 healthy controls (Cs).
Results
Patients with DA had *larger* gray matter volumes than did both the VOD cases and Cs in mostly right HVIIb-HVIII (Fig. 1a-c), whereas the VOD cases had *smaller* gray matter volume than did both the DA patients and the Cs in a partly overlapping cerebellar region (Fig. 1a, c-d). Also, compared to the Cs, the VOD cases had *smaller* gray matter volumes in I-VI bilaterally and in mostly right HVIIa Crus I (Fig. 1d), as well as *smaller* gray matter volume in the dentate bilaterally compared to both other groups (Fig. 1e).
Conclusion
We propose that the differential locus of the cerebellar abnormalities and their behavioural consequences in DA and VOD reflect the basic underlying cerebellar somatotopy.

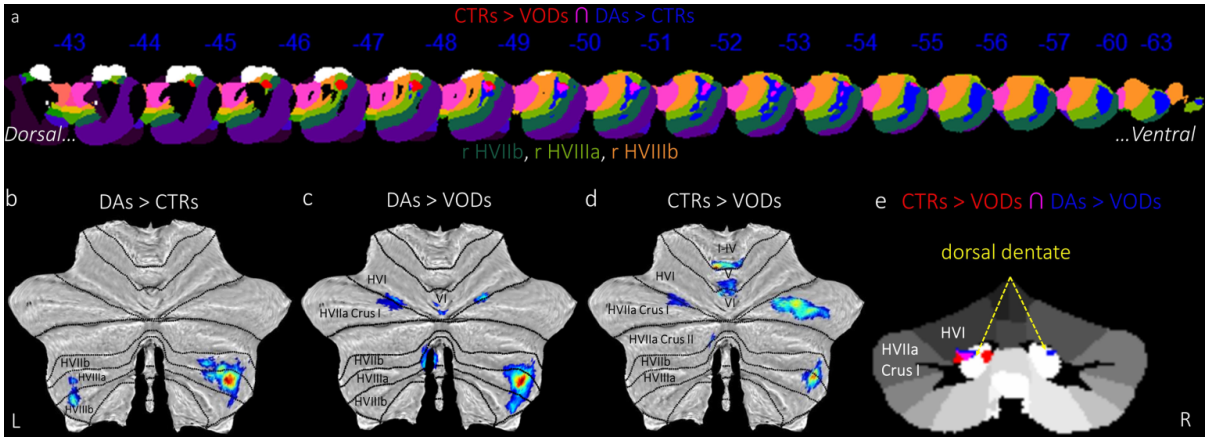

Fig.1. Cerebellar structural abnormalities in DA and VOD. Clusters survive corrections for non-stationary smoothness and FWE ($p < .05$); a: right serial axial slices; b-d: flatmaps; e: coronal slice, dentate.

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5. Yu, W., & Krook-Magnuson, E. *Front Syst Neurosci*, 9.177 (2015).

Disclosures: G.P. Argyropoulos: None. **K.S. Saleem:** None. **M. Mishkin:** None. **F. Vargha-Khadem:** None.

Poster

557. Motor and Sequence Learning

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Program#/Poster#: 557.25/MMM46

Topic: H.02. Human Cognition and Behavior

Support: NIH/NEI ROIEY024056 to LTL

Title: Brain reorganization and performance correlates of a rapid switch in handedness induced by drawing training in a left-handed blind individual

Authors: *L. T. LIKOVA, K. MINEFF, S. NICHOLAS;
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Abstract: The neural correlates of hand preference are still debatable, and there are only a few studies on the mechanisms of enforced change of handedness from left to right in early childhood (e.g., Grabowska et al., 2012). The question of retraining handedness in late adulthood well outside the accepted critical period for brain plasticity, has not been previously studied. To address this question we used a unique training on a complex task in a 50 y.o. habitually left-handed man, who became totally blind 10 years ago. In a week of 2 hr/day of Cognitive-Kinesthetic Drawing Training (Likova, 2012), the participant learned to draw freely faces and objects from non-visual memory with his non-dominant (right) hand. He had never been able to draw well even with his dominant (left) hand while still sighted, and was highly surprised by this outcome. An additional session focused on training to draw the already memorized images with his dominant (left) hand. Functional MRI was run before and after the training on a sequence of five tasks (20s each): tactile exploration of raised-line drawings of faces and objects (left hand only); tactile drawing from memory (right and left hand); and a scribble motor/memory control (right and left hand). As expected, drawing with the dominant (left) hand produced bilateral activation with contralateral (right) predominance, as did drawing with the non-dominant (right) hand before training, but with left predominance. After training, however, rapid functional reorganization was observed for the non-dominant (right) hand, leading to a strong unilateral activation in the left (contralateral) hemisphere with a massive suppression in all motor control related, somatosensory and respective superior parietal areas the right (ipsilateral) hemisphere, correlated with the dramatic enhancements in drawing performance. This study is the first to show that the non-preferred hand is not always controlled by both hemispheres as has been thought. Only a week of CK training was able to overturn 50 years of dominance of the right hemisphere in a complex memory-driven motor task. The data suggest a critical role for functional mechanisms such as inter-hemispheric competition as opposed to structural predetermination in hand dominance. Consistent with previous findings (Likova, 2012, 2013, 2014, 2015), they also demonstrate the power of the Cognitive-Kinesthetic Drawing Training to cause rapid and effective brain reorganization. The results will be further discussed in the context of the long-standing debate about the sources of hemispheric asymmetry in the cortical organization of motor function.

Disclosures: **L.T. Likova:** None. **K. Mineff:** None. **S. Nicholas:** None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

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Program#/Poster#: 557.26/MMM47

Topic: H.02. Human Cognition and Behavior

Title: Explicitly trained sequences display vulnerability to performance pressure due to monetary incentives while implicitly trained sequences do not

Authors: *T. G. LEE;
Univ. of Michigan, Ann Arbor, MI

Abstract: Although increased motivation usually enhances performance, impaired performance can sometimes be seen in situations involving large rewards and high motivational states. Over thirty years of psychological research has provided scientific evidence of the anecdotally salient phenomenon of this "choking under pressure". However, the cognitive mechanisms underlying choking remain unclear. One proposed theory of choking asserts that reduced performance is due to an increase in explicit monitoring of a procedural skill. Here we tested whether explicit skill knowledge is a necessary prerequisite for choking under pressure.

Participants were trained across eight different blocks on four separate 8-item motor sequences in the discrete sequence production (DSP) task. Crucially, for two of the sequences the identity of the upcoming sequence was explicitly cued with a colored square just prior to each trial and participants were told to attempt to learn these sequences. The other two (implicitly trained) sequences were given an ambiguous gray square just prior to each trial and participants were told that these sequences were randomized. The sequence order was actually randomized on one third of the trials cued with the gray cue in order to effectively mask awareness of the implicitly trained sequences.

Following training, participants were asked to perform the same task for reward bonuses with time limits set on an individual-by-individual basis based on movement times at the end of training. Each trial was associated with a $5, $10, or $20 incentive by a reward cue displayed just prior to the start of the trial. Participants were able to modulate their performance (as measured by accuracy) on explicitly trained sequences based on the reward values displayed, but displayed stereotypical choking behavior as indexed by reduced performance at the highest incentive level. However, accuracy on implicitly trained sequences was unaffected by incentive value and participants did not display impaired performance for the highest level of reward. These results suggest that skill metacognition can both positively and negatively impact performance in a feed-forward manner and that knowledge of expected ability in a motor skill can contribute to choking under pressure. Future work with fMRI scanning during the training and performance of explicitly cued sequences with varying levels of incentives will elucidate the neural mechanism by which this skill knowledge impacts the motor skill.

Disclosures: T.G. Lee: None.

Poster

557. Motor and Sequence Learning

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Program#/Poster#: 557.27/MMM48

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant K12HD055931

Title: Investigating the effect of motor state during paired associative stimulation on cortical excitability and motor skill performance

Authors: W. A. GRAY, A. C. KNOTT, P. E. O'SHEA, E. C. OETTER, K. WILDE, *M. R. BORICH;
Emory Univ., Atlanta, GA

Abstract: Introduction: Repeated pairing of electrical stimulation of a peripheral nerve with transcranial magnetic stimulation (TMS) over the primary motor cortex (M1) "hotspot" for a target muscle can induce short-term neuroplastic adaptations in the human brain. Currently, it is unclear if motor state of the targeted muscle during this form of paired associative stimulation (PAS) influences the induction of long-term potentiation (LTP)-like plasticity. Additionally, the effect of PAS on both motor skill acquisition and learning is not well characterized. Here, we investigated the effect of motor state during PAS on measures of cortical excitability, motor skill acquisition and motor skill retention. **Methods:** Twelve young, neurologically-intact healthy participants completed three separate visits separated by at least one week, each consisting of one of three PAS protocols: PAS_{REST} (no active contraction in the left abductor pollicis brevis [APB]), PAS_{ACTIVE} (10% maximal voluntary contraction [MVC] of the left APB), and $PAS_{CONTROL}$ (10% MVC of left APB paired with sham TMS). PAS consisted of 180 pairs of left median nerve stimulation (at APB motor threshold) followed by TMS over contralateral M1 (~1mV intensity), with an inter-pulse interval based on the individual N20 latency + 5ms delivered at 0.25Hz. Measures of cortical excitability (motor evoked potential [MEP] peak-to-peak amplitude) and motor skill performance (reaction time) on the serial reaction time task (SRTT) were collected prior to PAS, directly following PAS and at 30 and 60min post-PAS. Motor skill retention was indexed by SRTT performance at a delayed (>1wk) retention test. **Results:** Significant effects of motor state during PAS on measures of cortical excitability and SRTT skill acquisition were not detected. A trend for enhanced SRTT performance at retention testing after PAS_{REST} was observed. **Discussion:** Preliminary results suggest that motor state does not appear to differentially modulate responsiveness to PAS. Traditional PAS approaches may contribute to enhanced motor skill retention. However, inter-individual variability in response to PAS for each protocol restricts conclusions drawn from the group-level results. Evaluation of individual response profiles may provide additional information to guide future

PAS interventions. Subsequent studies should evaluate the impact of PAS on the time course of motor skill learning in healthy individuals and the recovery of motor function following neurological insult.

Disclosures: W.A. Gray: None. **A.C. Knott:** None. **P.E. O'Shea:** None. **E.C. Oetter:** None. **K. Wilde:** None. **M.R. Borich:** None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 557.28/MMM49

Topic: H.02. Human Cognition and Behavior

Support: Bial Foundation

Title: Can random number generation be taught implicitly?

Authors: *U. MAOZ, G. MERHOLZ;
Psychology, UCLA, Los Angeles, CA

Abstract: Human ability to be random, as the flipside of predictability, is important for various neuroscience fields, such as decision-making, volition, and theory of mind. So it is no surprise that it has drawn interest over the years. Most research finds human ability to be random lacking, with people underestimating the the chance likelihood of repeated events, for example. It is therefore probably that we could train human subjects to be more random by explicitly pointing out their deviations from randomness and asking them to correct those. But to what degree could humans be implicitly taught to be random?
To test this, we constructed a 3-part experiment. In all parts, subjects selected one of 3 options—rock, paper, or scissors—using the keyboard. In the first part of the experiment, we instructed subjects to generate a 100-long series of rock, paper, and scissors that would be as random as possible. They received no feedback on how random the series was. In the second part of the experiment, subjects played 100 trials of rock/paper/scissors against the computer. This time, they were told whether they won, tied, or lost. The computer used a prediction algorithm that searched for patterns in each subject's transitions between rock, paper, and scissors, taking into account also wins, ties, and losses. Thus, subjects' best strategy was to be as random as possible. Half the subjects were told of the algorithm, the others were not. In the third and last part of the experiment, subjects were once again instructed to generate a 100-long random series of rock, paper, and scissors, with no feedback. Before part 1 of the experiment, participants filled out a short questionnaire about their confidence and knowledge of randomness. After part 3, they

further filled out a short questionnaire about their experience and to gauged their own performance during the study. The objective was to test whether any learning that occurred during the game part also generalized to simple, non-competitive random sequence generation. We determined the degree of randomness of the sequences using tests for equiprobability of events outcome (one third each for rock paper and scissors); sequential independence (how well previous trials can predict the current trial); event symmetry; and repetition or repetition avoidance. Preliminary results suggest that some subjects are better able to create random sequences than others. There is also evidence that subjects do learn to become more random as the experiment progresses. We expect more control experiments to shed more light on the effect of implicit learning on random-sequence generation in humans.

Disclosures: **U. Maoz:** None. **G. Merholz:** None.

Poster

557. Motor and Sequence Learning

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 557.29/MMM50

Topic: H.02. Human Cognition and Behavior

Support: CONACYT-CVU606870

Title: Audiomotor learning: facilitation of implitic sequence learning through explicit melody memorization

Authors: *L. JIMENEZ-DABDOUB[1], P. KIRK[2], R. BODAK[3], L. STEWART[2];
[1]Lab. of Psychology and Musical Arts-Unam, Ciudad DE Mexico, Mexico; [2]Psychology, Goldsmiths, Univ. of London, London, United Kingdom; [3]Aarhus Univ., Aarhus, Denmark

Abstract: Goldsmiths audio-motor coupling research group guided by Stewart is beginning to underpin all research previously done focusing on musicians. Now, we are paying attention to non-musicians as it might help understand audio-motor learning and music in the general population. Motor performance for playing a melody has been demonstrated to improve without any physical practice only by listening to a previously practiced piano piece (Lahav et al, 2006). In 2015, Stephan and colleagues conducted a study about motor sequence memories by exposing participants to associate four finger key-pressing movements to four tones (implicit learning); and for them to learn either a melody (explicit learning) to be congruent or incongruent to the motor sequence they would be tested on. The expected effect was to facilitate implicit memorization of a motor sequence. The present study aimed to approach similar findings but instead of having participants pressing keys, a series of four hand gestures (HG) were performed.

HG were recorded by placing two accelerometers in participants' right thumb and middle fingers. "The beneficial effect of sound on motor performance has also inspired the idea of music-supported therapy (MST)" (Stephan et al., 2015: 319). Hence, by using real gestures, we expect to create a bridge towards the possible clinical applications of the facilitation effects music could have in re-learning daily living tasks. Overall, results showed no significant differences in the mean response time (mRT) between group A (Congruent condition) and Group B (incongruent condition). This study does provide a step into the use of new musical devices to support motor rehabilitation. It is suggested to explore and create a more congruent space-pitch mapped gestures and including a rhythmic component to focus at. On the other hand, testing over time and through a cross-over design, application from Stephan and colleagues research would thorough review, which is actually being done by Bodak as her PhD research.

Disclosures: L. Jimenez-Dabdoub: None. **P. Kirk:** None. **R. Bodak:** None. **L. Stewart:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 558.01/MMM51

Topic: I.04. Physiological Methods

Support: NINDS: 5U01NS090557-02

Title: A multi-channel, semi-chronic micromanipulator system for combined electrophysiological recording and optogenetic manipulation of neuronal activity in behaving, non-human primates.

Authors: *A. B. GOODELL, C. M. GRAY;
Gray Matter Res., Bozeman, MT

Abstract: In order to gain a greater understanding of the neural mechanisms that mediate cognitive function new approaches and technologies are needed to dramatically expand the ability to record and manipulate the activity of large numbers of neurons throughout widespread areas of the primate brain. To accomplish this objective, we have developed a multi-channel, semi-chronic micromanipulator system that permits the implantation of up to 32 independently movable optrodes in behaving non-human primates. This device is combined with a guide array for precisely controlled viral injections. Multiple microdrives can be implanted and flexibly configured to enable the long-term measurement and optogenetic manipulation of neuronal activity from distributed circuits spanning the depth and breadth of the brain.

The optrodes are fabricated by bonding a beveled optical fiber to a microelectrode with epoxy. Each assembly is then bonded to a non-round, threaded brass shuttle using conductive epoxy. Optrode position is controlled by rotation of a fixed leadscrew (125 μm/turn resolution) that advances or retracts the shuttle over travel distances up to 40 mm. The electrical signal from each electrode passes through the shuttle to the leadscrew and to a printed circuit board mounted on top of the microdrive. Each optical fiber is routed below the PCB to a custom connector assembly that is mounted on the actuator block of the microdrive. Sufficient slack is incorporated into the length of the optical fiber to account for the optrode travel. The actuator block, connector assembly and external housing are fabricated using 3D printing technology.
Ongoing work includes the design and testing of viral injection methods, the surgical removal of the dural membrane to facilitate the penetration of the delicate optrodes into the brain, and the incorporation of miniature, multi-channel optical fiber connectors. The completed system will enable precise spatio-temporal control of activity in distributed neuronal circuits.

Disclosures: **A.B. Goodell:** A. Employment/Salary (full or part-time): Gray Matter Research. **C.M. Gray:** A. Employment/Salary (full or part-time): Gray Matter Research.

Poster

558. Optogenetic Tools for Studying Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 558.02/MMM52

Topic: I.04. Physiological Methods

Support: NSF EBICS 0939511

NSF EAGER DBI 1450962

Title: Light-evoked responses from channel rhodopsin-expressing neuronal cells studied by quantitative phase imaging

Authors: T. KIM[1], M. SHAN[1], V. NASTASA[1], M. WANG[2,3], P. SENGUPTA[2,4], *M. U. GILLETTE[5,2,3,4], G. POPESCU[1,2];
[1]Dept. of Electrical and Computer Engin., [2]Beckman Inst. for Advanced Sci. and Technol., [3]Dept. of Mol. and Integrative Physiol., [4]Dept. of Bioengineering, Univ. of Illinois, Urbana, IL; [5]Dept. of Cell & Developmental Biol., Univ. Illinois, Urbana, IL

Abstract: Light stimulation of channelrhodopsin-2 (ChR2)-expressing neuronal cells causes cations to flow from outside to the cell interior thereby causing depolarization of the membrane and initiating a signaling cascade. These subtle and rapid events occur in millisecond to second

timescales, which makes it extremely difficult to detect them without an expensive electrophysiology system. Moreover, these systems also require a highly precise control during operation and are typically limited to single-point measurements. More recently, electrophysiology systems based on MEMS devices have been developed for multipoint detection of these signals. However, it remains challenging to acquire wide-field label-free imaging of these cells in action because of the required imaging speed and sensitivity. Recent advances in quantitative phase imaging (QPI) allow label-free live biological specimens to be imaged with sub-nanometer sensitivity and diffraction-limited resolution. A QPI technique called Spatial Light Interference Microscopy (SLIM) has been applied for studying neuronal network formation and dynamics and has proven its potential usefulness in neuroscience [Z. Wang et al., Opt. Exp., 19, 1016 (2011) & M. Mir et al., Sci. Rep., 4, 4414 (2014)]. This technique allows for wide-field imaging of live neuronal activities and can be used for measurement of rapid and subtle action potentials in optogenetically engineered cells. By implementing a projector and imaging a pattern onto the sample plane, we can excite individual ChR2-expressing cells with light with high spatial resolution. Using SLIM's label-free and wide-field imaging, the cellular signals generated as a response to this light stimulation are measured with a sub-nanometer sensitivity as they propagate through the cells. An increase in signal over a few seconds is measured from ChR2+ cells as a response to the blue light stimulation. On the other hand, no response was detected from ChR2- cells with blue light or from ChR2+ cells with red light stimulation. This new optical imaging technology offers the possibility for wide-field measurement of electrical events in neurons, and ultimately can help understand cellular signaling mechanisms post-stimulation and cellular dynamics at a higher resolution.

Disclosures: **T. Kim:** None. **M. Shan:** None. **V. Nastasa:** None. **M. Wang:** None. **P. Sengupta:** None. **M.U. Gillette:** None. **G. Popescu:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 558.03/MMM53

Topic: I.04. Physiological Methods

Support: NEI R01-EY024067

Simons SCGB 325548

Title: Local field potentials reflect excitability of frontal cortical neurons to optogenetic stimulation of posterior parietal cortex

Authors: *R. SHEWCRAFT, D. A. HAWELLEK, K. A. BROWN, B. PESARAN;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Population neuronal activity measured by the local field potential (LFP) may measure how well long-range synaptic input can drive local spiking due to changes in excitability. However, the need to causally control circuit activity while measuring its large-scale effects means this hypothesis has not been directly tested. We used large-scale recordings in frontal cortex (PFC and Brodmann areas 4 and 6) of awake monkeys to measure responses to optogenetic stimulation in posterior parietal cortex (Brodmann areas 5 and 7).
We implanted a 96-channel semi-chronic movable electrode array (SC96, Gray Matter Research) over the frontal lobe in two rhesus macaques and recorded single unit activity. We then injected AAV5-hSyn-ChR2(H134R)-EYFP (UNC Vector Core) into the superior and inferior parietal lobules of the PPC. We stimulated sites in PPC while simultaneously recording responses in PPC and across the frontal array. Stimulation of area 7 drove activity in prefrontal cortex. Stimulation of area 5 drove activity in premotor cortex.
We found 47 sites in frontal cortex with multi-unit activity (MUA) that was driven by parietal stimulation. For each MUA site, we measured how the phase of the frontal LFP varied with the probability of eliciting a spike in frontal cortex by computing the conditional probability of a spike occurring after stimulation given the phase at the time of stimulation. We found significant modulations in the alpha band (8-12 Hz) at 70% of sites (33/47, $p<0.05$), in the beta band (15-25 Hz) at 68% of sites (32/47, $p<0.05$) and in the gamma band (30-50 Hz) at 68% of sites (32/47, $p<0.05$). For each band, the preferred phase slightly preceded the LFP trough (334, 324 and 336 degrees, respectively), which would suggest that excitability peaks during periods of synchronized depolarization across the local neuronal population. We propose that the LFP in these bands measures the local sensitivity to synaptic inputs.
Does each frequency band independently modulate excitability, or are the alpha, beta and gamma bands correlated, providing a common mechanism for modulation? There was no significant difference in the proportion of modulated sites between bands ($p>0.05$, Fisher's exact test). In addition, we found that the phases at the time of stimulation were correlated (alpha-beta, 30% of sites; alpha-gamma, 36%; beta-gamma, 62%). While the extent to which neuronal excitability is reflected in LFP activity due to frequency-specific mechanisms remains unclear, our results demonstrate that frontal cortical LFP activity across a range of frequency bands reflects neuronal excitability to input from PPC.

Disclosures: R. Shewcraft: None. **D.A. Hawellek:** None. **K.A. Brown:** None. **B. Pesaran:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

Location: Halls B-H

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Program#/Poster#: 558.04/MMM54

Topic: I.04. Physiological Methods

Support: Welcome Trust

ERC

BBSRC

MRC

Title: Closed-loop real-time all-optical interrogation of neural circuits *In vivo*

Authors: *Z. ZHANG, L. E. RUSSELL, A. M. PACKER, M. HAUSSER;
Wolfson Inst. for Biomed. Res., Univ. Col. London, London, United Kingdom

Abstract: Since neural activity patterns are rapidly evolving and highly variable from trial to trial, it is crucial to be able to read out and interfere with these patterns in real time. Such closed-loop interventions would provide direct causal links between specific activity patterns and behaviour. Recently we described an all-optical method for circuit interrogation where populations of neurons are simultaneously recorded and addressable with single-cell resolution (Packer et al 2015). Here we close the loop between readout and stimulation by implementing a real-time feedback module, which consists of hardware and software tools to allow rapid configuration of stimulation patterns. We use this module to demonstrate dynamic, activity-guided circuit manipulations. Populations of neurons in mouse visual cortex coexpress GCaMP6s and C1V1 to permit fast two-photon calcium imaging and optogenetic stimulation respectively. A high-speed spatial light modulator enables precise photostimulation of multiple cells and rapid updates of the stimulation pattern. Real-time analysis of the raw data stream from the imaging channel is made possible with custom-written software. We then implement various types of feedback control logic to enable closed-loop manipulation of target populations. Here we demonstrate three categories of proof-of-principle experiments. First, we optically monitor single or multiple cells. Upon event detection we near-instantaneously trigger photostimulation to pre-defined targets. Second, we introduce ‘activity clamp’, whereby single and multiple cells are clamped at particular fluorescence levels through continuous on/off feedback control. Third, we demonstrate a conjunction of feedback control with sensory stimulation. Weak sensory stimulation can be selectively, optogenetically boosted if sensory-evoked activity fails to pass threshold. Our closed-loop approach will ultimately enable new classes of experiment, such as: correcting erroneous network trajectories recorded from animals performing decision-making

tasks; inducing plasticity in neural circuits by recruiting cells into functional ensembles; and processing functional connectivity maps online to update predictions and guide subsequent interrogation.

Disclosures: Z. Zhang: None. **L.E. Russell:** None. **A.M. Packer:** None. **M. Hausser:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 558.05/MMM55

Topic: I.04. Physiological Methods

Title: Simultaneous multiwell optogenetic stimulation and microelectrode array recording for disease modeling and toxicological assays

Authors: I. P. CLEMENTS, *H. B. HAYES, A. M. NICOLINI, C. A. ARROWOOD, D. C. MILLARD, J. D. ROSS;
Axion Biosystems, Atlanta, GA

Abstract: Microelectrode arrays (MEAs) monitor and manipulate cultured cell activity *in vitro*, providing insight into neuronal network interactions to inform "disease-in-a-dish" models, stem cell characterization, toxicology screening, and drug safety and development. Recently-developed multiwell MEA systems (48, 96, or more MEA wells on a single microplate) enable high-throughput assessment of functional endpoints at greatly reduced time and cost. While network activity may be monitored under spontaneous conditions, stimulation of neural activity allows evaluation of evoked activity measures, reduces variability across wells, reduces assay duration by increasing activity levels, and enables creation of application specific protocols to assess features of network connectivity. Optogenetics integrates fast, light-activated channels (opsins) that allow targeted, precise manipulation of cellular activity, and provides advantages such as targeting of specific cell types, the ability to suppress activity, minimal stimulus artifact, and uniform stimulus delivery across a culture. Here, we demonstrate the application of Lumos, a commercial multiwell optical stimulation system, to enhance typical assays in toxicology screening and disease-in-a-dish models through optogenetic stimulation with Channelrhodopsin-2 (ChR2). These findings demonstrate the potential of optically-integrated multiwell MEA systems to enable high-throughput drug screening and phenotypic modeling of neurological diseases.

Disclosures: I.P. Clements: A. Employment/Salary (full or part-time): Axion Biosystems. **H.B. Hayes:** A. Employment/Salary (full or part-time): Axion Biosystems. **A.M. Nicolini:** A.

Employment/Salary (full or part-time): Axion Biosystems. **C.A. Arrowood:** A. Employment/Salary (full or part-time): Axion Biosystems. **D.C. Millard:** A. Employment/Salary (full or part-time): Axion Biosystems. **J.D. Ross:** A. Employment/Salary (full or part-time): Axion Biosystems.

Poster

558. Optogenetic Tools for Studying Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

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Topic: I.04. Physiological Methods

Support: Bertarelli Foundation

Med-El

NIH/NIDCD grant F31 training grant (A.E.H)

Title: Targeted optogenetic stimulation in the auditory pathway enables access to the tonotopic axis

Authors: *S. NARASIMHAN[1], A. HIGHT[2], X. MENG[3], A. EDGE[5], C. BROWN[6], D. LEE[4]; [1]Eaton Peabody Laboratory, Otolaryngology, Mass Eye and Ear Institute/ Harvard Med. Sch., Boston, MA; [2]Speech and Hearing Biosci. and Technol., Harvard Med. Sch. / Eaton Peabody Labs., Boston, MA; [3]Eaton Peabody Laboratories/ Otolaryngology, [4]Otology and Otolaryngology, Mass Eye and Ear Infirmary/ Harvard Med. Sch., Boston, MA; [5]Eaton Peabody Laboratories/ otolaryngology, Massachusetts Eye and Ear Institute/ Harvard Med. Sch., Boston, MA; [6]Eaton Peabody Laboratory/ Otolaryngology, Massachusetts Eye and Ear Infirmary/ Harvard Med. Sch., Boston, MA

Abstract: Aim: The auditory brainstem implant (ABI) restores hearing sensations to individuals who are deaf and cannot benefit from a cochlear implant(CI) due to a damaged or abnormal auditory nerve or cochlea. Speech comprehension of ABI subjects is lower compared to those of CI subjects. A likely limiting factor of ABI is electrical current spread, which indiscriminately stimulates both excitatory and inhibitory neurons of the cochlear nucleus (CN), impeding the delivery of resolved frequency cues. We are exploring whether optogenetics, activating neurons with light instead of electricity, might provide activation of populations of auditory neurons carrying frequency specific cues. For each population, we test whether activation of the CN provides for frequency specific activation of response areas in the inferior colliculus (IC). So far, we have used 5 unique transgenic mouse lines.

Methods: Cell specific expression of opsins is obtained by crossing floxed ChR2 mice with the following 5 cre lines: Bhlhb5, Parvalbumine, Atoh1, Nestin and Vglut2. Surgical exposure of the CN is performed acutely in the transgenic mice. Blue light pulses are delivered by a diode laser at 28pulses/s. Light beams are focused with an adjustable laser collimator and beam location is shifted along the rostral-caudal and medio-lateral axes of CN. Multiunit activity is recorded from the IC using a single-shank,16-site recording probe placed along its tonotopic axis. Placement is confirmed with acoustic tones. Auditory brainstem responses (ABR) are also measured. The center of evoked activity in the IC with respect to stimulation location on CN is used to evaluate access to the tonotopic axis. Post-experiment histology is performed confirming opsin expression in the CN.
Results: Histology demonstrated unique patterns of opsin expression for each transgenic line. Light stimulation of the DCN evokes excitatory responses across the tonotopic axis in the IC for all lines except Vglut II, which exhibited inhibitory response patterns. Parvalbumin and Bhlhb5 lines, produce frequency specific evoked neural activity in the IC whereas the broadly expressed Nestin and Atoh1 produce nonspecific responses. Optically evoked ABRs (oABRs) depend on the neuronal populations stimulated in the CN.
Conclusion: Our experiments reveal that evoked responses depend on the type of neuronal population stimulated, exhibiting excitatory or inhibitory responses. This suggests that the selection of a promoter or transcription factor for targeting cell-specific opsin expression is essential for an optically-based ABI.

Disclosures: S. Narasimhan: None. **A. Hight:** None. **X. Meng:** None. **A. Edge:** None. **C. Brown:** None. **D. Lee:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 558.07/MMM57

Topic: I.04. Physiological Methods

Title: Optogenetic, transient-sensing and chemogenetic mouse models available from The Jackson Laboratory.

Authors: *J. BECKWITH, S. ROCKWOOD, M. SASNER;
The Jackson Lab., Bar Harbor, ME

Abstract: The Jackson Laboratory (JAX) Mouse Repository distributes mouse lines with optogenetic and transient-sensing (calcium-, voltage-, glutamate-) technologies. Opsins are light-activated proteins that alter membrane potential in neurons, so that stimulation with light allows

rapid control of neuronal activity. Several mouse lines express improved/optimized opsins fused to fluorescent proteins. These include mice with channelrhodopsin expression directed by specific promoters. Additional control is available in mice with Cre- or Tet-dependent expression of channelrhodopsin or halorhodopsin variants.
Variants of GCaMP fluoresce in response to calcium binding and serve as an indicator of neuronal activation. These include the Thy1-promoter driven GCaMP3, GCaMP6f or GCaMP6s transgenic lines, the Tet-dependent GCaMP6f or GCaMP6s transgenic lines and the Cre-dependent GCaMP3, GCaMP5, GCaMP6f or GCaMP6s mouse lines.
Several strains utilize both Cre-lox and Tet-On/-Off functionality. Removal of a floxed-STOP allows Tet-dependent expression of channelrhodopsin (Chronos/EGFP), halorhodopsin (Jaws/EGFP), GCaMP6s, GCaMP6f or FRET chromophores sensitive to calcium (YCX2.60), voltage (VSFPB1.2) or glutamate (iGluSnFR).
This set features models created by the Allen Institute for Brain Science, the Genetically-Encoded Neuronal Indicator and Effector (GENIE) Project (Janelia/HHMI), Duke/MIT and several others. Transgenic lines from the Cornell Heart Lung Blood Resource for Optogenetic Mouse Signaling (CHROMus) are designed for combinatorial crosses enabling the coexpression of sensors and effectors, or red and green calcium sensors (*e.g.*, RCaMP and GCaMP8).
Designer receptors exclusively activated by designer drugs (DREADDs) are mutant G-protein coupled receptors activated by the pharmacologically-inert molecule clozapine-N-oxide. Several chemogenetic strains have Cre-, Tet- and/or FLP-inducible expression of M3Dq, M4Di or M3Ds.
The JAX Mouse Repository - a centralized facility for the development, rederivation, cryopreservation and distribution of mouse models to the international biomedical research community - adds hundreds of new strains annually. Repository holdings may be searched online (JAXMice database: jax.org/mouse-search). Researchers may donate their mouse strains using an online submission form (jax.org/donate-a-mouse). Please visit The Jackson Laboratory Resources for Optogenetics, Cre-dependent Optogenetic Tools and Cre Strains for Neurobiology (jax.org/optogenetics). The JAX Mouse Repository is supported by NIH, The Howard Hughes Medical Institute and several private charitable foundations.

Disclosures: J. Beckwith: None. **S. Rockwood:** None. **M. Sasner:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 558.08/MMM58

Topic: I.04. Physiological Methods

Support: CIHR

Title: Multi-colour optogenetic manipulation of cAMP and cGMP signalling from synapse to circuits

Authors: *M. VALENCIA[1,2], T. T. LUYBEN[1,2], K. OKAMOTO[1,2];
[1]Lunenfeld-Tanenbaum Res. Inst., Mount Sinai Hosp., Toronto, ON, Canada; [2]Mol. Genet., Univ. of Toronto, Toronto, ON, Canada

Abstract: Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are major intracellular signaling molecules that are modulated both positively and negatively and in connection with each other. In neurons, cAMP and cGMP play important roles in synaptic plasticity, learning and memory, however the precise details of their dynamic interaction are not fully known. To study the role of cAMP and cGMP interaction at the synapse, we established an optogenetic strategy to selectively activate cAMP and cGMP signalling using different colours of light.

For the multi-colour optogenetic approach, we validated the codon-optimized form of a bacterial photoactivatable adenylyl cyclase (PAC) and its mutant guanylyl cyclase (BlgC), which produce cAMP and cGMP, respectively, in response to blue light. To combine with these photoactivatable enzymes, we prepared and examined a far-red light-sensitive adenylyl (NIR-PAC) and guanylyl cyclase (NIR-PGC), and also a bacterial rhodopsin-guanylyl cyclase (RhGC) which is sensitive to green light. These photoactivatable enzymes were rapidly activated in the milliseconds order with the relevant wavelength of light and then inactivated upon its removal *in vitro*. We found that PAC and RhGC can be used together to selectively activate cAMP and cGMP with two different colours of light, enabling the precise regulation of their timing-dependent interaction.

To target the photoactivation at the single synapse level, we have used PAC or BlgC with two-photon laser light excitation. To expand this technique for multi-colour optogenetics using different wavelengths of excitation light, we tested the activity of each enzyme across the two-photon excitation spectra *in vitro.* We found that longer wavelengths of two-photon light (>1,000 nm) efficiently photoactivated RhGC and far-red light sensitive enzymes (NIR-PAC, NIR-PGC), while shorter wavelengths (up to 1,000 nm) well-activated the blue light-sensitive enzymes such as PAC.

These approaches allow non-invasive spatiotemporal manipulations of cAMP and cGMP signalling from target synapse to entire brain regions, enabling systematic analysis of their interactive function. We will discuss how these optogenetic technologies will serve as valuable tools for elucidating the role of cAMP and cGMP in living neurons.

Disclosures: **M. Valencia:** None. **T.T. Luyben:** None. **K. Okamoto:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 558.09/MMM59

Topic: I.04. Physiological Methods

Support: CIHR

Title: Optogenetic control of phosphodiesterase activity in living neurons

Authors: *F. BERGIN, K. OKAMOTO;
Lunenfeld-Tanenbaum Res. Inst., Toronto, ON, Canada

Abstract: Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) are second messengers with a variety of essential physiological functions including synaptic plasticity which is critical for learning and memory. Phosphodiesterases (PDEs) are the enzymes that degrade cAMP and cGMP providing critical regulatory control of these second messengers and have been actively targeted for drug discovery for a variety of psychiatric and neurodegenerative disorders. However, the activity-dependent dynamics and pre-synaptic roles of cAMP and cGMP signaling are poorly understood due to a lack of methods available to selectively and spatiotemporally manipulate these second messengers. To overcome this limitation, we report optogenetic tools to manipulate PDEs activity by light to specifically degrade cAMP and cGMP. We designed photoactivatable PDE4 and PDE5 to hydrolyze cAMP or cGMP using a far-red light sensitive domain PAS-GAF-PHY and the catalytic domain of PDEs (Gasser *et al*, 2014). Upon milliseconds of applied LED light, photoactivatable PDE4 and PDE5 showed strong cAMP or cGMP degradation, demonstrating their rapid photoactivation and catalytic specificity *in vitro*. Furthermore, the photoactivatable PDEs demonstrated a rapid inactivation upon removal of light. PDEs exist as membrane bound or soluble forms, which results in important subcellular regulation of cAMP and cGMP signaling. In addition to the soluble photoactivatable enzymes, we created membrane bound photoactivatable PDE4 and PDE5 utilizing a membrane localization signal. The membrane bound photoactivatable PDEs also have similar light-dependent activity and localized to the cellular membrane in living neurons. For activation of photoactivatable PDEs at the target single synapse level in living tissues, we utilized two-photon microscopy. We examined the two-photon excitation spectra of photoactivatable PDEs *in vitro* to understand the optimal wavelength to activate each PDE. We found the longer two-photon excitation wavelengths (more than 1,000 nm) corresponding to the far-red light sensitive domain efficiently activated these PDEs *in vitro*, suggesting the ability to control its activation at single synapses. We will discuss their application in *in vivo* studies from synapse to brain level.

Disclosures: F. Bergin: None. **K. Okamoto:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 558.10/MMM60

Topic: I.04. Physiological Methods

Title: *In vivo* application of an integrated multi-beam nanophotonic switch for high resolution optogenetic stimulation

Authors: *Q. LI[1], A. MOHANTY[2,3], M. A. TADAYON[2,3], M. LIPSON[2,3], A. KEPECS[1]; [1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Dept. of Electrical Engin., Columbia Univ., New York, NY; [3]Sch. of Electrical and Computer Engin., Cornell Univ., Ithaca, NY

Abstract: The ability to optically turn on or off the activity of specific neural populations has revolutionized the investigation of neural circuits. Most applications, however, employ a single optical fiber to flood a large area of the brain with light, limiting the ability to selectively activate neurons with high spatial resolution in vivo. Using silicon photonics technology it is possible to miniaturize complex optical circuits on a silicon chip, resulting in a quantum leap in optical control, matching the scale and resolution currently achieved by electrical silicon probes for electrical recordings.

Here we developed and tested the first visible light multipoint-emitting nanophotonic switches for in vivo optogenetics. To enable selective activation of neurons using multiple, independently controllable optical beams, we used micro-switches that could route light toward different diffraction gratings. Fast and independent control over each beam was achieved via external micro-controllers. We demonstrated that the same technology previously used for switching light in the near infrared spectral range could be used for switching light in the visible, using the thermo-optic effect of silicon nitride, a material that is transparent to the visible spectral range. To characterize the probe performance in mammalian brain in vivo, anaesthetized mice expressing ChR2 in PV+ inhibitory interneurons (PV-Cre x Ai32 mice) is used. The demonstrated probe employs a 1x4 multipoint-emitting nanophotonic switch (emitter size: 20 x 20 μm, 250 μm interbeam distance) coupled to a tungsten electrode array. The probe could be readily inserted and lowered into cortex. By applying two external microcontrollers to arbitrarily direct 472nm blue light to 4 output grating light emitters we could independently activate single ChR2-expressing PV interneurons throughout layers 2-6 of visual cortex in vivo. The activated PV interneurons showed robust spike firing with short first spike latency (1.475 +/- 0.063 ms, mean +/- sem) and low jitter (0.031 +/- 0.003 ms, mean +/- sem). In conclusion we successfully show our ability to use external microcontrollers to arbitrarily direct light to multiple densely spaced illumination points along the shank. Scaling it up to a larger number of beams (e.g.128) is expected to be straightforward since these devices are defined lithographically, enabling high-resolution optical stimulation in deep brain regions.

Disclosures: **Q. Li:** None. **A. Mohanty:** None. **M.A. Tadayon:** None. **M. Lipson:** None. **A. Kepecs:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

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Program#/Poster#: 558.11/MMM61

Topic: I.04. Physiological Methods

Title: Precision optogenetic stimulation using directional light from a multichannel optrode

Authors: ***J. J. WHEELER**[1], J. REGISTER[2], C. SEGURA[2], J. LEBLANC[2];
[1]Draper Lab., Boston, MA; [2]Draper Lab., Cambridge, MA

Abstract: In this work, we evaluate a method of light steering in small volumes of nerve tissue for optogenetics techniques and present a microfabricated device for the purpose. Optical simulations were carried out using Monte Carlo techniques with consideration to both scattering and absorption using the Henyey-Greenstein and Beer-Lambert models, respectively. The parameters of tissue volume, emission geometry, emitter size, and wavelength were varied in the simulation study. Data from the analysis was then used to drive the design of multimode waveguide structures made from photo-patternable polymers with the goals of minimizing both bending radii and insertion loss for compact geometries. Structures were fabricated using standard UV lithography with careful consideration to materials selection. The work has shown how multiple light emitters could be used to selectively activate small regions of tissue when greater spatial discrimination is needed.

Disclosures: **J.J. Wheeler:** None. **J. Register:** None. **C. Segura:** None. **J. LeBlanc:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

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Program#/Poster#: 558.12/MMM62

Topic: I.04. Physiological Methods

Support: NIH Grant 1U01NS094190

Title: A fully integrated optrode based on multipoint emitting tapered optical fibers: the "fibertrode"

Authors: *L. SILEO[1], M. PISANELLO[1], B. L. SABATINI[2,3], M. DE VITTORIO[1], F. PISANELLO[1];
[1]Inst. Italiano di Tecnologia, Arnesano, Italy; [2]Dept. of Neurobio., Harvard Med. Sch., Boston, MA; [3]Howard Hughes Med. Inst., Boston, MA

Abstract: We present a new technological paradigm to realize fully integrated optrodes for simultaneous multipoint optogenetic control of neural activity and multisite single unit extracellular recording. The device, composed by a multipoint emitting tapered optical fiber (MPF) [Pisanello et al, Neuron 82, 1245 (2014)] along with multiple electrodes for extracellular recording, is realized by means of combined focused ion beam induced deposition and milling. The result is a fully integrated optrode (hereafter referred to as "fibertrode") with a tip cross-section <1μm and allowing for multipoint control and monitor of neural activity simultaneously. The technology is based on multiple focused ion beam milling and ion beam induced deposition processes performed on the MPF covered with alternating layers of metal and insulator thin films. The main advantage of the approach is the possibility to obtain from the same multilayered optical fiber a geometrical combination of photo-stimulation volumes and electrical recording sites which is tailored from time to time to the actual target region to be investigated. Moreover, points of illumination and points of electrical recording can be realized at any location on the non-planar surface of the taper, in contrast to other approaches in which the technology used constraints their arrangement on a plane configuration [Neuron 86, 106 (2015)]. In this study, the reliability for use in acute experiments of microelectrodes realized with the proposed technology is first demonstrated in vitro by testing their impedance-vs-time characteristics in saline physiological solution, which gave results comparable with those of commercial Michigan arrays. In order to exemplify the versatility of the approach, a number of different configurations were then realized with one, two or three microelectrodes combined with different optical windows geometries (a big single window or multiple apertures), with microelectrodes and optical windows fabricated on the same face or on opposite faces of the taper surface. Microelectrode performances were independent on the number, type and placement of the optical windows. Spontaneous activity was successfully recorded in head restrained mice validating the presented technology for its acute use in vivo and opening the way to experiments of open-loop and closed loop recordings realized with minimally invasive and fully integrated fibertrodes designed to suit the topography of the target region.

Disclosures: **L. Sileo:** None. **M. Pisanello:** None. **B.L. Sabatini:** None. **M. De Vittorio:** None. **F. Pisanello:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

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Program#/Poster#: 558.13/NNN1

Topic: I.04. Physiological Methods

Support: W.M. Keck Foundation

NIH Grant MH093412

Title: Shielded coaxial optrode arrays

Authors: ***M. J. NAUGHTON**[1], J. R. NAUGHTON[2], M. J. BURNS[2], T. CONNOLLY[2], J. A. VARELA[2], J. A. VARELA[2], J. M. MERLO[2], T. C. CHILES[2], J. P. CHRISTIANSON[2]; [1]Dept. of Physics, [2]Boston Col., Chestnut Hill, MA

Abstract: Recent progress in the study of the brain has been greatly facilitated by the development of new tools capable of minimally-invasive, robust coupling to neuronal assemblies. Two prominent examples are the microelectrode array, which enables electrical signals from large numbers of neurons to be detected and spatiotemporally correlated, and optogenetics, which enables the electrical activity of cells to be controlled with light. In the former case, high spatial density is desirable but, as electrode arrays evolve toward higher density and thus smaller pitch, electrical crosstalk increases. In the latter, finer control over light input is desirable, to enable improved studies of neuroelectronic pathways emanating from specific cell stimulation. Here, we introduce a coaxial electrode architecture that is uniquely suited to address these issues, as it can simultaneously be utilized as an optical waveguide and a shielded electrode in dense arrays. Using optogenetically-transfected cells on a coaxial microelectrode array, we demonstrate the utility of the shielded architecture by recording cellular currents evoked from optical stimulation. We also show the capability for network recording by radiating an area of individually-addressed coaxial electrode regions with cultured cells covering a section of the extent.

Disclosures: **M.J. Naughton:** None. **J.R. Naughton:** None. **M.J. Burns:** None. **T. Connolly:** None. **J.A. Varela:** None. **J.A. Varela:** None. **J.M. Merlo:** None. **T.C. Chiles:** None. **J.P. Christianson:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

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Program#/Poster#: 558.14/NNN2

Topic: I.04. Physiological Methods

Support: NIH R01MH094730

HFSP RGP0052/2012

Title: Tetherless magnetothermal deep brain neuro-stimulation and -silencing to modulate behavior in awake, unrestrained mice

Authors: *A. PRALLE, R. MUNSHI, I. CASTELLANOS-RUBIO, S. QADRI;
Physics, Univ. At Buffalo, SUNY, Buffalo, NY

Abstract: Precise remote, tetherless stimulation of deep brain circuits evoking specific behaviors in unrestrained subjects would facilitate neuroscience research of brain circuits underlying behavior and emotion. Using bioconjugated superparamagnetic nanoparticles and a heat-sensitive cation-channel, we demonstrate how the energy of magnetic fields can be transduced into evoking robust locomotion in awake, freely moving mice. Stimulation in the motor cortex evoked ambulation, deep brain stimulation in the striatum caused rotation around the body-axis, and even deeper stimulation near the ridge between ventral and dorsal striatum caused freezing of gait. The animals respond within seconds of field onset, and the behavior persists for the duration of the field. By combining the magnetic nanoparticle heating with a heat-sensitive chloride channel, we cause remote, magneto-thermal silencing of neurons. In a place preference assay, we show that this remote silencing can in-vivo modulate behavior of freely moving mice. Membrane binding of the nanoparticles allows for high spatial control with minimal nanoparticle volumes. The presented approach provides genetically and spatially targetable activation of neuro-circuits deep inside the brain without any tether into the brain or any surgical implantation of any device.

Disclosures: **A. Pralle:** None. **R. Munshi:** None. **I. Castellanos-Rubio:** None. **S. Qadri:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

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Topic: I.04. Physiological Methods

Support: U01 NS090565-01

R01 NS083875-02

R01 NS065110-05

Title: Key residues in fluorescent protein reverses the polarity of voltage sensitivity in the voltage indicator ArcLight

Authors: *J. PLATISA[1,2], G. VASAN[1,2], V. A. PIERIBONE[1,2];
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Abstract: Genetically encoded calcium indicators (GECIs) produce unprecedentedly large signals which make them the only indicators to allow routine optical recording of single neuron activity *in vivo* in rodent brain. Genetically encoded voltage indicators (GEVIs) offer a more direct measure of neuronal electrical status, however the signal to noise characteristics and signal polarity of the probes developed to date have precluded routine *in vivo* use. We applied directed evolution to target areas of the fluorescent protein in the GEVI ArcLight to create the first GFP-based GEVI (Marina) that exhibits a $\Delta F/\Delta V$ with a positive slope relationship. We found that only three rounds of site-directed mutagenesis produce a family of 'positively going' GEVIs with voltage sensitivity comparable to that seen in the parent indicator ArcLight. Voltage sensitivity and good membrane localization of Marina allows for detection of single action potentials and subthreshold events in spontaneously active mammalian neurons *in vitro.* This shift in signal polarity is an essential first step to producing voltage indicators with signal to noise characteristics comparable to GCaMPs and support widespread use *in vivo.*

Disclosures: J. Platisa: None. **G. Vasan:** None. **V.A. Pieribone:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

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Topic: I.04. Physiological Methods

Support: Canadian Institutes for Health Research FDN143209

Canadian Partnership for Stroke Recovery

Leduc Foundation

Title: Mouse homecage self directed motor learning and brain imaging: training, software, and hardware.

Authors: F. BOLANOS[1], G. SILASI[1], J. D. BOYD[1], J. LEDUE[1], S. H. SCOTT[2], *T. H. MURPHY[1];
[1]Univ. British Columbia, Vancouver, BC, Canada; [2]Queens, Kingston, ON, Canada

Abstract: Automated assessment of rodent behaviour is desired as it reduces experimenter bias, facilitates high throughput, avoids disrupting circadian rhythms and eliminates stress introduced by experimenter handling. We developed an automated mouse lever pulling task that mice can perform 24 hrs a day in the home cage to assess forelimb function (see image with 4 mice in cage). The apparatus consists of a small chamber attached to the outside of the home cage, which contains a recessed lever that the mice can access with their forelimb. The lever is attached to the axle of a rotary encoder (or torque motor) allowing us to measure lever position during each pulling event. An RFID sensor on the roof of the training compartment detects a unique RFID tag that is implanted subcutaneously in each mouse at the nape of the neck. To encourage task participation, mice are water restricted, and a solenoid attached to a drinking spout dispenses a ~10µl drop of water upon the successful completion of a lever pull. In the initial phase of training mice receive a water reward for just entering the training compartment, while subsequent phases require the mice to hold the lever in a rewarded position for incrementally longer durations and a smaller range of movement. Individualized performance is logged and task parameters are controlled by custom software written in Python running on a Raspberry Pi keeping costs low and the footprint small. Mice enter the training chamber ~300 times in a 24 hr period and perform over 500 trials once they have acquired the task. The number of successful trials increased during the first week of training, and on average it takes ~7 days of unsupervised training for mice to acquire a 1 second hold duration on the lever. Future experiments will introduce perturbations to lever position during the hold period to assess properties of voluntary motor control. We are exploring adding the sensory-motor lever task to similar cages that

support automatic head-fixation and brain imaging (Murphy et al. 2016 Nature Commun.) to permit automated brain circuit assessment in the context of the forelimb task.

Disclosures: **F. Bolanos:** None. **G. Silasi:** None. **J.D. Boyd:** None. **J. LeDue:** None. **S.H. Scott:** None. **T.H. Murphy:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

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Program#/Poster#: 558.17/NNN5

Topic: I.04. Physiological Methods

Support: NMU Foundation

Title: Effects of optogenetic activation of ventral tegmental area neurons on locomotor activity and temperature in TH-Cre rats treated with amphetamine or raclopride

Authors: ***R. RICE**[1], Z. MESSER[2], S. WHITEHOUSE[2], E. N. OTTEM[3], A. J. PRUS[2]; [1]Psychology, Virginia Commonwealth Univ., Benzonia, MI; [2]Psychology, [3]Biol., Northern Michigan Univ., Marquette, MI

Abstract: The mesolimbic dopamine pathway is an important structure to study for understanding the rewarding effects of drugs of abuse and for elucidating the neurotransmitter mechanisms of mental disorders, such as schizophrenia. The recent development of optogenetic techniques provides a high precision approach for evaluating behavior following selective stimulation of mesolimbic dopamine neurons. The present study sought to examine the behavioral effects of a dopamine agonist and antagonist during activation of mesolimbic dopamine neurons. Male TH-Cre rats were used to express Channelrhodopsin-2 (ChR2), through viral mediated gene deliver, in the ventral tegmental area (VTA). ChR2 was activated using 465nm (blue) light via fibers installed dorsal to the VTA, which led to a significant increase in locomotor activity in an open field and body temperature compared to when the light was deactivated. Amphetamine (i.p.) significantly increased locomotor activity to a far greater extent than light activation in saline-treated rats; the effect of amphetamine did not differ between the activation or deactivation of light. Amphetamine also did not increase body temperature compared to saline. The dopamine $D_{2/3}$ receptor antagonist raclopride did not attenuate light-induced increases in locomotor activity or body temperature, although only a small sample size was available during this phase of the study. As expected, these results demonstrate that selective activation of mesolimbic dopamine neurons enhances locomotor activity and body temperature, although amphetamine produced a much greater increase in locomotor activity than light. These findings may correspond to differences in dopamine release elicited by enhancing the firing rate of dopamine neurons versus the effects of amphetamine at axon terminals. The lack of inhibition by raclopride of light-induced locomotor activity may either be due to a low sample size or may indicate that other receptors, such as D_1 receptors, may mediate these effects.

Disclosures: **R. Rice:** None. **Z. Messer:** None. **S. Whitehouse:** None. **E.N. Ottem:** None. **A.J. Prus:** None.

Poster

558. Optogenetic Tools for Studying Neural Circuits

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Program#/Poster#: 558.18/NNN6

Topic: I.04. Physiological Methods

Support: DARPA Cooperative Agreement W911NF-14-2-0107

NIH Grant F32NS092430

Title: Virus, opsin, and immunomodulation selection for optogenetic control of peripheral motor function

Authors: *J. J. WILLIAMS[1], A. L. VAZQUEZ[2], C. WIRBLICH[4], M. J. SCHNELL[4], A. B. SCHWARTZ[3];
[1]Systems Neurosci. Inst., [2]Dept. of Radiology, [3]Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [4]Dept. of Microbiology and Immunol., Thomas Jefferson Univ., Philadelphia, PA

Abstract: The incorporation of light sensitive opsins into peripheral motor nerves offers a promising alternative to traditional functional electrical stimulation in restoring lost motor function. Gene therapy to express such optogenetic constructs for functional optical stimulation of muscles has been successfully demonstrated in non-transgenic rodents. However, the procedure for transducing opsins into peripheral motor nerves is technically difficult, and only a limited catalog of proven constructs in this context exists to date. To realize a robust clinical outcome for this approach, it will be necessary to demonstrate expression in primate peripheral nerves which has not yet been achieved. In light of this need, our present study examines the efficacy of several viral vectors and opsins in transducing nerves sensitive to functional optical stimulation. Here, we discuss ongoing experiments and results with several AAV-based vectors (serotypes 6 and 9) as well as a non-replicating rabies virus (NRRV) vector. In addition, we present results from constructs utilizing one of two opsins: the traditional channel rhodopsin (ChR2) as well as the more recently described Chronos. Finally, we will describe our protocol and results for administering dexamethasone as an immunosuppressant to facilitate virus transduction into motor nerve cells. Hopefully, our findings will provide valuable insight into approaches that will translate well to primate peripheral motor gene therapy.

Disclosures: J.J. Williams: None. **A.L. Vazquez:** None. **C. Wirblich:** None. **M.J. Schnell:** None. **A.B. Schwartz:** None.

Poster

559. Anatomical Techniques: Tomography

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Topic: I.03. Anatomical Methods

Support: the National Basic Research Program of China （973 Program）granted No.2015CB755603

Title: Chemical sectioning: high throughput imaging brain networks *Ex vivo* at synaptic resolution

Authors: *S. ZENG[1], H. XIONG[1], X. WANG[1], Y. GANG[1], T. YANG[1], L. SU[1], A. LI[1], S. JIN[2], Z. SHANG[1], Y. JIA[1], K. HUANG[1], X. LV[1], S. LI[1], Y. LI[1], N. LI[1], L. LIU[1], T. XU[1], F. XU[2], H. GONG[1], Q. LUO[1];
[1]Huazhong Univ. of Sci. & Technol., HB, China; [2]Wuhan Inst. of Physics and Mathematics, Chinese Acad. of Sciences, Wuhan, China, Wuhan, China

Abstract: The complex anatomical structures of individual neurons and their synaptic connections form the signal transmission and processing pathway of the nervous system, and therefore are basis for understanding brain functions. However, existing imaging methods always need to compromise the speed, and signal-to-noise ratio and thus is difficulty to cover the huge extension of neurons in mammalian brain with a resolution sufficient to identify connection sites (the pre- and postsynaptic structures). Here we proposed a so-called chemical sectioning (CS) method, a tomographic imaging method, that enables systematically reconstruct the integral morphology of individual neurons, even across a whole-mouse-brain, at synaptic resolution. With the help of sparse labeling, for the first time, we show the successful reconstruction of individual neurons extending axonal projections throughout the brain, including all dendrites, centimeter-extended axonal main stems, terminal arborizations, intensive dendritic spines and axonal boutons, and putative synapses. Our method enables quantitative analysis of the morphology, projections, and connectivity of genetically defined neurons. This method will shed light for neuroscience studies like cell type, neural circuits and neuronal computations.

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Poster

559. Anatomical Techniques: Tomography

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Topic: I.03. Anatomical Methods

Support: NIH Grant R01AG039452 to BVZ

NIH Grant R01DC010856 to RAF

Title: Serial two-photon tomography for three-dimensional vasculature reconstruction of mouse cochlea

Authors: *D. LAZIC[1,3], K. KISLER[1], A. AHUJA[1], P. SALEHI[2], P. S. TSAI[4], R. A. FRIEDMAN[2], B. V. ZLOKOVIC[1];
[1]Physiol. and Biophysics, [2]USC Tina and Rick Caruso Dept. of Otolaryngology-Head & Neck Surgery, USC, Los Angeles, CA; [3]Neurobio., Inst. for Biol. Research, Univ. of Belgrade, Belgrade, Serbia; [4]Physics, Univ. of California, San Diego, CA

Abstract: Understanding the spatial distribution of various cell types that occurs in physiological and pathological conditions is of great importance. Although many two-dimensional immunostaining techniques are widely used to reveal cellular architecture in different organs, many cell distribution patterns, such as vasculature, are not readily appreciated in two-dimensions. Serial two-photon (STP) tomography is a powerful technique that combines automated serial sectioning and two-photon microscopy imaging of fluorescently labeled tissue throughout the whole organ, providing three-dimensional (3D) high resolution datasets down to sub-micron level. Normal functioning of cochlea is dependent on adequate blood supply and disruption of cochlear vasculature and blood flow appear to be linked to hearing impairment. Studying of the mouse cochlear vascular network and blood flow is challenging due to its small size, complex spiral shape, and location deep in the inner ear, making it poorly accessible for *in vivo* imaging studies. Here, we describe a method using STP tomography to generate 3D vascular reconstruction map of the mouse cochlea. We injected six week-old C57BL/6 mice with DyLight 594-conjugated tomato lectin in superficial femoral vein to label perfused blood vessels. Following paraformaldehyde perfusion, the cochlea was dissected and subjected to further fixation, decalcification and clearing steps. Whole cochlea was covalently mounted in agarose and scanned on STP tomography. Vascular density, diameter and length were analyzed using customized Volumetric Image Data Analysis (VIDA) MatLab script. Overall, the use of this novel STP tomography method and analysis of vasculature network offers a relatively straightforward and fast assessment of the mouse inner ear vascular architecture. In addition, this approach can address many important questions regarding vascular organization in different conditions such as aging, noise exposure and ototoxicity, all of which can contribute to hearing loss.

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Poster

559. Anatomical Techniques: Tomography

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Program#/Poster#: 559.03/NNN9

Topic: I.03. Anatomical Methods

Support: Texas Institute for Brain Injury and Repair (TIBIR)

Title: Circuit mapping in mouse models of brain injury and disease by serial two-photon tomography

Authors: *D. M. RAMIREZ[1], A. HERNANDEZ[2], J. A. BIBB[2,1,3], M. P. GOLDBERG[1], J. P. MEEKS[4];
[1]Neurol. and Neurotherapeutics, [2]Dept. of Psychiatry, [3]Harold C. Simmons Comprehensive Cancer Ctr., [4]Dept. of Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Serial two-photon tomography (STPT), a technique that produces large-scale, three-dimensional images of intact brain tissue, is expanding the capacity to investigate brain-wide circuit mechanisms associated with neurological and psychiatric disorders as well as brain injury. The UT Southwestern Whole Brain Microscopy Facility (WBMF) (http://www.utsouthwestern.edu/education/medical-school/departments/neurology/research/microscopy-facility/index.html), part of the newly established Peter O'Donnell Jr. Brain Institute, is coordinating research efforts by several laboratories that are investigating the circuit basis of brain disorders across the micro-, meso-, and macro-scales of inquiry. The facility houses two TissueCyte 1000 STPT microscopes capable of producing high-resolution (minimum voxel size less than 1 μm^3) images of entire rodent brains subjected only to standard perfusion fixation procedures in preparation for imaging. Here, we report progress towards imaging and analyzing region- and cell-type specific projections in mouse brains. We have now optimized sample preparation and imaging conditions for a variety of tissues, including adult brain, early postnatal brain and spinal cord. Submitting laboratories used several techniques to introduce fluorophores into neurons of interest, including anterograde and retrograde fluorescent dyes, anterograde and retrograde viral injections, and transgenic mice that express Cre recombinase in a cell type-specific manner. To probe the changes in regional and cell type-specific projections that occur after various nervous system perturbations, STPT-based circuit tracing was used in conjunction with genetic models of neuropsychiatric disease and experimentally-induced brain injury. Preliminary results across several experimental paradigms establish the utility of this approach for identifying circuit-level dysfunction in complex brain disorders. Future work will incorporate computational strategies for automated analyses of features of interest in whole brain datasets (e.g. cell body quantification, axon tract length). We ultimately hope to apply our facility's large-scale, high-throughput model for brain-wide connectivity analysis to identify therapies that improve neural circuit connectivity in models of neuropsychiatric and neurodegenerative disorders.

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Poster

559. Anatomical Techniques: Tomography

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Topic: I.03. Anatomical Methods

Support: FRQNT

NSERC Discovery

CIHR

Title: Serial OCT scanner : comparing 3D histology to *In vivo* imaging in the entire mouse brain

Authors: *A. CASTONGUAY, J. LEFEBVRE, P.-L. TARDIF, P. DELAFONTAINE-MARTEL, P. POULIOT, F. LESAGE;
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Abstract: In the study of brain anatomy and function, histological examination remains the gold standard to fully comprehend in vivo measurements. For this purpose, a serial OCT scanner was designed to image large 3D sections of biological tissues at microscopic resolution. OCT imaging reveals changes in refractive indices, giving rise to high contrast between white and grey matter in the mouse brain. METHODS: Serial imaging of mice brains embedded in agarose blocks was performed by cutting through brain tissue using a vibratome. The vibratome sequentially cut slices in order to reveal new tissue to image, overcoming the limited light penetration encountered in optical microscopy of biological tissues. Raw data was then post-processed to stitch each individual acquisition and obtain a reconstructed volume of the brain. To validate the ability of the scanner to provide a reliable representation of the in vivo brain, 4 wild type mice were used. The brains were first imaged in vivo with a 7 Tesla MRI scanner. Mice were then sacrificed, perfused with 4% PFA and re-imaged with the MRI scanner to assess deformations caused by fixation. Finally, brains were extracted, fixed in agarose and imaged using the serial OCT scanner. Template brains for each imaging modality (MRI in vivo and ex vivo, OCT scanner) were obtained using Advanced Normalization Tools (ANTs), which was also used to measure morphometric changes between imaging modalities. For each modality, whole brain volume, cortical volume, white matter vs grey matter ratio and deformation fields between modalities were analysed. RESULTS: Preliminary results show little variation in whole brain volume between MRI in vivo (343,3 ± 5,8 mm^3) and ex vivo brains (344.0 ± 15.3), suggesting little deformation caused by perfusion. The ratio of cortical volume over whole brain volume remained constant between every modality (MRI invivo : 27,4 ± 1,2 %, MRI ex vivo : 25,2 ± 2,1% , slicer : 26,5 ± 0.7 %). CONCLUSION: Serial microscopic scanners proved to be a novel massive histological imaging technique able to accurately represent in vivo measurements.

This proof of principle study will pave the way for further work requiring histological analysis in the whole brain and correlation to in vivo measurements.

Disclosures: **A. Castonguay:** None. **J. Lefebvre:** None. **P. Tardif:** None. **P. Delafontaine-Martel:** None. **P. Pouliot:** None. **F. Lesage:** None.

Poster

559. Anatomical Techniques: Tomography

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 559.05/DP10 (Dynamic Poster)

Topic: I.03. Anatomical Methods

Support: NIH U01MH105971

NIH R01MH096946

Title: OPT: light sheet fluorescence microscopy with increased spatial resolution

Authors: ***A. NARASIMHAN**, K. UMADEVI VENKATARAJU, D. F. ALBEANU, P. OSTEN;
Cold Spring Harbor Lab., Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: We have designed a custom microscope, called Oblique Plane Tomography (OPT), that operates on the principle of light sheet fluorescence microscopy (the sample is illuminated with a scanned thin light beam to generate a planar light sheet and images are acquired by wide-field fluorescence microscope placed perpendicular to it), but the illumination/detection paths are oriented obliquely (45°) with respect to the tissue surface. This oblique orientation allows for imaging the surface of the brain to ~400 μm depth in an XY raster pattern. Once the raster scan is completed, the brain is translated to an integrated vibratome to section the imaged tissue. The raster scan and automated sectioning is repeated iteratively to obtain whole brain coverage. Since tissue scattering limits the light penetration depth, we modified and optimized a clearing protocol based on the CUBIC protocol for clearing the brains [1]. Based on the current instrument configuration, a cleared adult mouse brain is imaged within ~10 hrs at 0.4 X 0.4 X 5 μm voxel resolution with overlapping regions for image registration and reconstruction. We use this custom built OPT to understand the connectivity of local and long-range individual neurons at single-cell resolution in the olfactory system. It is known that mitral cells (MTs) from a single glomerulus project randomly in the piriform cortex, but project to spatially distinct regions in the cortical amygdala [2]. What is not known, however, is the projection and spatial distribution of individual MTs to other regions of the brain. We use a sparse labeling approach using Sindbis

virus to trace the projections of the MTs in the whole brain. Additionally, to address the question as to whether there is any target-area specification at the level of single MTs to anatomically distinct regions such as piriform cortex or cortical amygdala, we use a reterograde targeting using CAV-DIO-mCherry, in each of the target areas to identify populations of cells that do and do not send collaterals. These viral approaches will elucidate the role of MT's in olfactory information processing.

Reference:
1. Susaki, E.A., et. al (2014) Cell, 157, 726-739.
2. Sosulski, D.L., Lissitsyna Bloom, M., Cutforth, T., Axel, R., Datta, S.R. (2011) Nature, 472, 213-219.

Disclosures: **A. Narasimhan:** None. **K. Umadevi Venkataraju:** None. **D. F. Albeanu:** None. **P. Osten:** None.

Poster

559. Anatomical Techniques: Tomography

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Topic: I.03. Anatomical Methods

Support: Science Fund for Creative Research Group of China #6142106

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NSF of Hubei Province #2015CFB448

Title: Generation of whole brain atlas for the cholinergic system via fluorescence micro-optical sectioning tomography

Authors: ***X. LI**[1], B. YU[2], Q. SUN[1], M. REN[1], Y. ZHANG[1], J. YUAN[1], Q. LUO[1], H. ZENG[3], H. GONG[1], Z. QIU[2];
[1]Wuhan Natl. Lab. For Optoelectronics, Hubei, China; [2]Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China; [3]Allen Inst. for Brain Sci., Seattle, WA

Abstract: The cholinergic system in the brain plays crucial roles in regulating motor functions and cognitive behaviors by modulating neuronal excitability. The 3-dimensional atlas of cholinergic neurons in the whole brain scale would be valuable to further understand the cholinergic system. Here we report the generation of the comprehensive whole-brain atlas for

cholinergic neurons by fluorescence micro-optical sectioning tomography (fMOST). With ChAT-IRES-Cre mice and Cre-dependent reporter line, cholinergic neurons were genetically fluorescent labeled, while almost all GFP-labeled subcortical neurons were choline acetyltransferase immunoreactive cells. We acquired the complete distribution of these GFP labeled neurons across different brain regions after reconstructing the whole-brain map precisely, including the characterization of intrinsic cholinergic cells of the cerebral cortex. Furthermore, we reconstructed the morphology of cholinergic neurons in the cortex, which showed layer-specific difference. These analyses provide a foundation for further functional dissection of the cholinergic system in the brain.

Disclosures: **X. Li:** None. **B. Yu:** None. **Q. Sun:** None. **M. Ren:** None. **Y. Zhang:** None. **J. Yuan:** None. **Q. Luo:** None. **H. Zeng:** None. **H. Gong:** None. **Z. Qiu:** None.

Poster

559. Anatomical Techniques: Tomography

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Program#/Poster#: 559.07/NNN12

Topic: I.03. Anatomical Methods

Support: 5R01NS092474

Title: Systematic comparison of ultrastructural and antigenic preservation of cortical tissue embedded in Lowicryl HM20 and LR White resins

Authors: ***M. M. NAUGLE**[1], K. N. PARKER[1], S. M. DAVIS[1], R. SERAFIN[1], F. COLLMAN[1], S. J. SMITH[1], R. J. WEINBERG[2];
[1]Synapse Biol., Allen Inst. For Brain Sci., Seattle, WA; [2]Dept. of Cell Biol. and Physiol., Univ. of North Carolina, Chapel Hill, NC

Abstract: There is a well-known trade-off between ultrastructural and antigenic preservation in plastic-embedded tissue. Here we compare two acrylic resins, LR White (generally considered especially effective for maintaining antigenicity) with Lowicryl HM20 (reputed to provide particularly good ultrastructure). After verifying these impressions of “reciprocal” performance for the two resins, we hypothesized that it might be possible to gain benefits offered by each resin by preparing mixtures. We find that LR White and Lowicryl are fully miscible; importantly, the mixtures polymerize effectively in the cold with UV light into blocks suitable for ultrathin sections. We used immunofluorescence on ultrathin section arrays to evaluate antigenicity, and scanning electron microscopy to examine ultrastructural preservation. Based on

initial promising results, we are investigating a wider range of mixtures for both antigenic and ultrastructural preservation.

Disclosures: **M.M. Naugle:** A. Employment/Salary (full or part-time): Allen Institute for Brain Science. **K.N. Parker:** None. **S.M. Davis:** None. **R. Serafin:** None. **F. Collman:** None. **S.J. Smith:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stephen Smith acknowledges a founder's equity interest in, and serves on the scientific advisory board of, Aratome, LLC, a Menlo Park CA startup commercializing array tomography goods and services. **R.J. Weinberg:** None.

Poster

559. Anatomical Techniques: Tomography

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Program#/Poster#: 559.08/NNN13

Topic: I.03. Anatomical Methods

Support: NIH NINS/NIMH 1R01NS092474 (TRA)

Title: Storing and analyzing large array tomography datasets in the cloud

Authors: ***A. D. BADEN**[1], F. COLLMAN[2], K. LILLANEY[1], J. T. VOGELSTEIN[1], R. BURNS[1];
[1]Computer Sci., Johns Hopkins Univ., Baltimore, MD; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: As automated methods for array tomography data collection improve, a multi-terabyte dataset can now be collected in as little as a few days. Specialized software is required to be able to store, visualize, and analyze this data. For example, the NeuroData Project currently hosts 100+ terabytes of public data spread across its cloud and local storage platforms. In this poster, we detail our current data processing pipeline with respect to array tomography. Using tools developed in conjunction with the NeuroData team, our pipeline supports storing multi-terabyte datasets in the cloud (NDStore), on-demand analysis and visualization of multi-channel datasets in the web browser (NDViz), and associating metadata with images using a rich and generalizable metadata framework (RAMON). Users can also download and upload data and run analysis tasks using ndio, our Python interface to NDStore services. Our data processing pipeline is briefly summarized as follows. First, 3D volumes (i.e. imaging tiles that have been stitched, aligned, and registered) are ingested into the NDStore spatial database. NDStore is optimized for quick, localized access to specific spatial regions. NDStore also enables the use of several additional services. For example, the NDStore histogram service allows the user to generate a

histogram using a background process. Once the histogram is generated, users can compute statistics across the entire dataset using only the histogram. We demonstrate several use cases, including setting a rough intensity range for converting 16-bit images to 8-bit images, and doing semi-automated quality control across a large number of imaging sessions. After ingest, data is available for use in our other web-based tools. NDViz supports dynamic visualization, maximum intensity projections, brightness / contrast image processing, and on-demand false coloring of multiple channels in the web browser. NDViz also supports dynamic, clickable metadata and annotation overlays. For example, we demonstrate uploading and visualizing the results of a synapse detection algorithm on an Array Tomography dataset using NDStore and NDViz. Our web tools also support creating links to specific views, allowing us to share data with collaborators by sending a hyperlink instead of a multi-terabyte volume.
Finally, we discuss next steps, including integrating an automated volume reconstruction pipeline, which would piece together the individual tiles into a stitched, registered, and aligned volume.

Disclosures: **A.D. Baden:** None. **F. Collman:** None. **K. Lillaney:** None. **J.T. Vogelstein:** None. **R. Burns:** None.

Poster

559. Anatomical Techniques: Tomography

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Program#/Poster#: 559.09/NNN14

Topic: I.03. Anatomical Methods

Support: NIH NINS/NIMH 1R01NS092474 (TRA)

NIH

DoD

Title: Query based probabilistic synapse detection in immunofluorescence data

Authors: ***A. K. SIMHAL**[1], C. AGUERREBERE[1], F. COLLMAN[2], J. T. VOGELSTEIN[3], K. D. MICHEVA[4], R. J. WEINBERG[5], S. J. SMITH[2], G. SAPIRO[1];
[1]Electrical Engin., Duke Univ., Durham, NC; [2]Synapse Biol., Allen Inst. for Brain Sci., Seattle, WA; [3]Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [4]Mol. and Cell. Biol., Stanford Univ. Sch. of Med., Stanford, CA; [5]Cell Biol. and Physiol., Univ. of North Carolina, Chapel Hill, NC

Abstract: The systematic examination of synaptic organization within large (> 1 mm^3) modules of brain containing millions of synapses requires robust techniques for synapse detection. Electron microscopy (EM) is the gold standard modality for synapse detection, but data acquisition is much slower and more expensive than with immunofluorescence (IF). The introduction of array tomography enables acquisition of hyperspectral proteometric data with IF, allowing identification of multiple synapse subtypes. This creates a need for effective synapse detection algorithms suitable for IF-only data.

The molecular diversity of synapses makes creating a canonical synapse model very challenging. Traditional machine learning techniques need large amounts of labeled training data for each potential subclass, which is often not available. Therefore, synapse detection algorithms must incorporate synaptic proteometric diversity and neuroscience knowledge.

We present a query-defined probabilistic synapse detection algorithm. For each excitatory synapse subtype, the user selects synaptic markers and synapse punctum sizes to consider. The output is a probability map; each voxel is the probability that it belongs to a synapse. This model-based algorithm incorporates fundamental biological knowledge of synapses and how they are manifested in the immunofluorescence data, providing detections with confidence values.

We evaluated the proposed approach on two conjugate array tomography datasets (for which synaptic identification had been confirmed with EM). Using six different query structures, we detected ~93% of PSD-95-expressing excitatory synapses, a level of accuracy comparable to a human experts. This fundamental approach opens the door to data-driven discovery of new synapse types and their density in a probabilistic fashion. The methods developed for this application are readily applicable to other datasets. All data, code, and data derivatives will be made available after publication through the NeuroData website, neurodata.io.

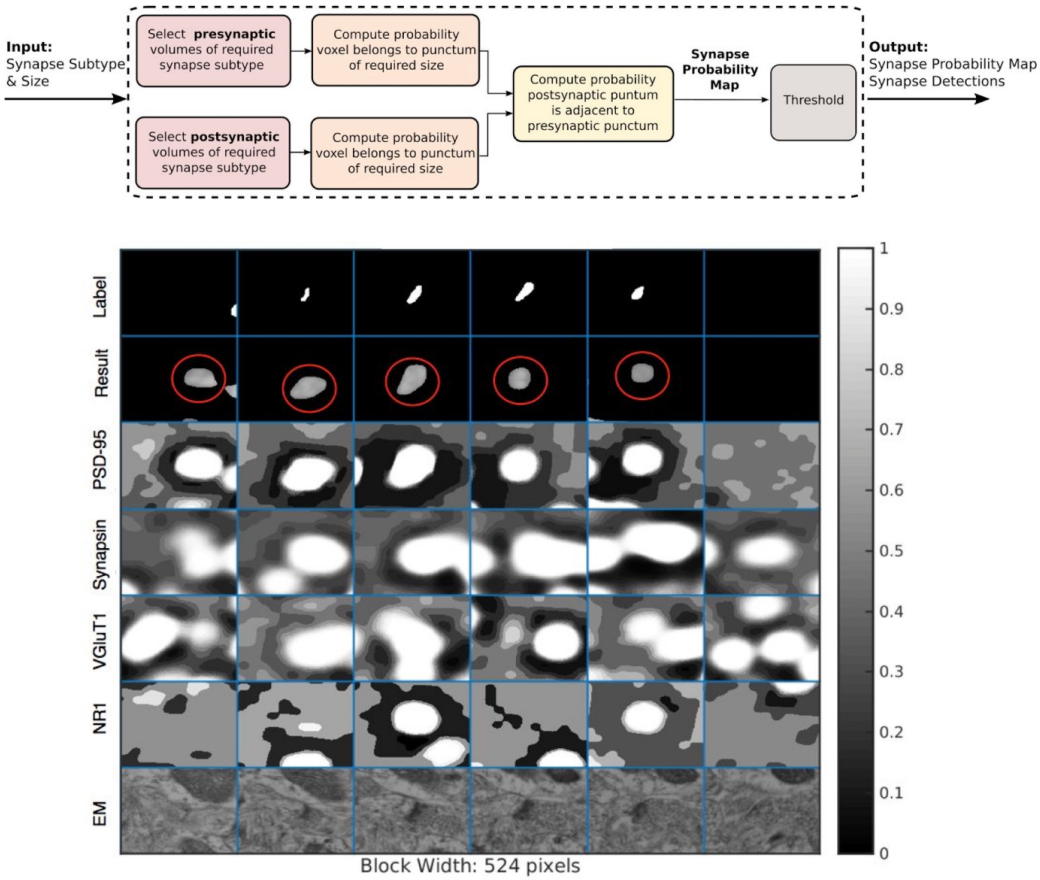

Figure 1. Top: Fundamental steps of the probabilistic synapse detection algorithm. **Bottom:** Example of a synaptogram. Each row is a channel and each column is a slice. Slices are spaced 70 nm apart and each pixel in a "window" is 2.33 nm x 2.33 nm. The number of pixels is given underneath the axis. 'Label' refers to the expert manual annotation of the synaptic cleft. 'Result' is the probabilistic output of the proposed synapse detection algorithm. The synapse detection is circled in red.

Disclosures: A.K. Simhal: None. **C. Aguerrebere:** None. **F. Collman:** None. **J.T. Vogelstein:** None. **K.D. Micheva:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); KDM has founder's equity interests in Aratome, LLC (Menlo Park, CA). KDM is listed as inventors on two US patents regarding array tomography methods. . **R.J. Weinberg:** None. **S.J. Smith:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SJS has founder's equity interests in Aratome, LLC (Menlo Park, CA). SJS is listed as inventor on two US patents regarding array tomography methods. . **G. Sapiro:** None.

Poster

559. Anatomical Techniques: Tomography

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Program#/Poster#: 559.10/NNN15

Topic: I.03. Anatomical Methods

Support: NIH NINS/NIMH 1R01NS092474 (TRA)

Title: ndparse: tools and interfaces for scalable neuroscience discovery

Authors: *W. R. GRAY RONCAL[1,5], J. MATELSKY[2], M. PEKALA[5], D. M. KLEISSAS[5], J. T. VOGELSTEIN[3,4], G. D. HAGER[2];
[2]Computer Sci., [3]Biomed. Engin., [4]Inst. for Computat. Med., [1]Johns Hopkins Univ., Baltimore, MD; [5]Res. and Exploratory Develop., JHU Applied Physics Lab., Laurel, MD

Abstract: The field of structural connectomics seeks to generate high-resolution maps of the brain, across various sensor modalities and scales ranging from mesoscale to nanoscale. The number of data challenges and algorithms is exploding, and users must decide on a processing pipeline without an easy way to explore alternatives and integrate new methods. However, many pipelines follow a predictable pattern: begin with a collection of images, align and stitch into a volume, format for compliance with a database or filesystem, process using computer vision and machine learning, and upload the result.
We introduce ndparse, a software toolkit that provides common interfaces and best practices for many of the use cases neuroimagers face when trying to extract knowledge from their data. We have written thin layers around popular tools and developed new protocols and algorithms to facilitate the generation of results in a standard, modular format. We include support for alignment and stitching (e.g., terastitcher), membrane detection (e.g., keras, theano), neuron segmentation (e.g., gala), object detection (e.g., ilastik, vesicle), post-processing, evaluation, and knowledge extraction.
ndparse is flexible and extensible to new algorithms, and it can be used with a variety of schedulers and meta-schedulers. Our interfaces facilitate reproducible and interoperable science and enable rapid prototyping and optimization. The community is invited to develop new functionality by submitting pull-requests at github.com/neurodata/ndparse. ndparse is compatible with ndio [github.com/neurodata/ndio], which greatly simplifies data access with local files or remote endpoints (e.g., NeuroData, DVID, S3).
We demonstrate the utility of this package via a use-case for graph generation in EM data. In this framework we show that work that previously required many lines of custom code can be run with only a few lines of descriptive python, black-boxing the underlying algorithms and simplifying the computational expertise required for scalable scientific discovery.

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Poster

559. Anatomical Techniques: Tomography

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NIH Grant R01MH104227

Paul and Jody Allen

Title: Fast, automated array imaging for synaptomics and connectomics

Authors: ***S. J. SMITH**, O. GLIKO, R. SERAFIN, S. SESHAMANI, M. NAUGLE, K. PARKER, S. DAVIS, K. LEPAGE, H. PENG, J. TING, E. LEIN, F. COLLMAN;
Synapse Biol., Allen Inst. For Brain Sci., Seattle, WA

Abstract: This poster describes an automated high-throughput array tomography platform designed to image cubic-mm-scale volumes of brain tissue (such as cortical physiology slices) by both multi-channel super-resolution fluorescence and electron microscopy. Fluorescence imaging of tomography arrays at a rate of 10,000 4.2-megapixel tiles per hour has been demonstrated and full automation of stain/wash/strip cycles is expected to allow unattended accumulation of 20 or more distinct fluorescence channels. Array tomography also allows for voxel-conjugate scanning electron microscopy (SEM) of selected array sub-regions after fluorescence imaging. Software now under development will allow for the automation of SEM sub-region selection driven by machine vision analysis of prior fluorescence images. We'll also describe progress toward automated image quality control and pre-processing software designed to enable robust streaming of fully restored fluorescence and SEM image tiles into the cloud for volume reconstruction, analysis and storage.
This array tomography platform is expected to image the entirety of mm3 volumes with 100 nm isotropic, multichannel voxels in a matter of days, with conjugate SEM imaging of thousands of sub-regions acquired in similar time periods. With use of appropriate antibodies targeting synaptic and cell-type-specific proteins, such image data will allow quantitative analysis of diverse synapse populations in mouse and human cortical tissue at the single-synapse level. In specimens where sparse subsets of neurons are labeled by fluorescent dyes or proteins,

reconstruction of complete neuronal arbors and their associated synaptic connections is expected to yield rich "shotgun" samples of cortical local circuit connectivity.

Disclosures: **S.J. Smith:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aratome. **O. Gliko:** None. **R. Serafin:** None. **S. Seshamani:** None. **M. Naugle:** None. **K. Parker:** None. **S. Davis:** None. **K. Lepage:** None. **H. Peng:** None. **J. Ting:** None. **E. Lein:** None. **F. Collman:** None.

Poster

559. Anatomical Techniques: Tomography

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Topic: I.03. Anatomical Methods

Support: NIH Grant NS019123

Title: Generation and validation of monoclonal antibodies for array tomography

Authors: ***J. S. TRIMMER**[1], B. GONG[1], K. D. MURRAY[1], K. D. MICHEVA[2], F. COLLMAN[3], M. M. NAUGLE[3], R. J. WEINBERG[4], S. J. SMITH[3];
[1]Neurobiology, Physiol. & Behavior, Univ. of California, Davis, Davis, CA; [2]Cell. and Mol. Physiol., Stanford Sch. of Med., Stanford, CA; [3]Allen Inst. for Brain Sci., Seattle, WA; [4]Cell Biol. & Physiol., Univ. of North Carolina, Chapel Hill, Chapel Hill, NC

Abstract: Highly validated antibodies that exhibit robust and specific immunoreactivity form the core of many neuroscience research projects. However, in many cases the immunoreactivity of antibodies can be profoundly influenced by sample preparation conditions, such that each antibody needs to be validated for efficacy and specificity in the specific application and sample preparation conditions in which it will be used. Array tomography (AT) is a technique that allows for an unprecedented combination of high resolution and deep proteomic dimensionality for subcellular localization of large sets of proteins in brain, features particularly relevant to the characterization of large populations of mammalian brain synapses. In general, sample preparation for AT has aspects distinct from those used to validate antibodies for use in other applications. As such, the efficacy and specificity of any given antibody for use in brain AT must be empirically determined, and in many cases available antibodies that have demonstrated efficacy and specificity in other applications are not useful for immunolabeling of brain AT samples. We have undertaken a concerted effort to both validate large sets of existing monoclonal antibodies, and to develop and validate novel monoclonal antibodies, with a unique

focus on directly assaying their efficacy and specificity in AT. We have also evaluated which of the most commonly used non-AT antibody validation procedures exhibit the highest value for predicting which monoclonal antibodies will exhibit efficacy and specificity in brain AT sections. These studies will allow for the development of a substantial set of renewable reagents that are available to the research community for use in applications employing immunolabeling of brain AT samples.

Disclosures: **J.S. Trimmer:** None. **B. Gong:** None. **K.D. Murray:** None. **K.D. Micheva:** None. **F. Collman:** None. **M.M. Naugle:** None. **R.J. Weinberg:** None. **S.J. Smith:** None.

Poster

559. Anatomical Techniques: Tomography

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Topic: I.03. Anatomical Methods

Support: P41-EB015896

P01-NS055104

R01-EB019956

AFOSR (MFEL FA9550- 07-1-0101)

Title: Imaging human brain cytoarchitecture with quantitative optical coherence tomography

Authors: ***D. A. BOAS**, C. MAGNAIN, H. WANG, E. KONUKOGLU, B. FISCHL, S. SAKADZIC;
Martinos Ctr. Biomed Imaging, Harvard Med. Sch., Charlestown, MA

Abstract: No current imaging technology allows us to directly and without significant distortion visualize the microscopic and defining anatomical features of the human brain. *Ex vivo* histological techniques can yield exquisite planar images, but the cutting, mounting and staining that are required components of this type of imaging induce distortions that are different for each slice, introducing cross-slice differences that prohibit true 3D analysis. We are overcoming this issue by utilizing Optical Coherence Tomography (OCT) with the goal to image whole human brain cytoarchitectural and laminar properties with potentially 3.5 μm resolution in block-face without the need for exogenous staining. From the intrinsic scattering contrast of the brain tissue, OCT gives us images that are comparable to Nissl stains, but without the distortions introduced in standard histology as the OCT images are acquired from the block face prior to slicing and

thus without the need for subsequent staining and mounting. We have shown that laminar and cytoarchitectural properties of the brain can be characterized with OCT just as well as with Nissl staining. A current limitation is that we presently integrate the signal over more than 50 μm in the axial direction to achieve sufficient contrast. We will present our recent advances to improve the axial resolution while maintaining contrast. Improvements are being obtained by developing speckle reduction procedures. In addition, we are beginning to obtain quantitative maps of the optical scattering coefficient, an intrinsic property of the tissue. We hypothesize that these quantitative maps will improve our contrast to the structural features of the brain and permit more isotropic resolution mapping of the volumetric features, enabling for example, the automated segmentation of neurons from multiple cm^3 of human brain tissue.

Disclosures: **D.A. Boas:** None. **C. Magnain:** None. **H. Wang:** None. **E. Konukoglu:** None. **B. Fischl:** None. **S. Sakadzic:** None.

Poster

560. Optical Methods: Real-Time Imaging

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 560.01/NNN19

Topic: I.04. Physiological Methods

Title: Functional imaging of VIP interneuron activity in behaving mice

Authors: *Z. SZADAI[1], H. PI[2], G. KATONA[3], T. TOMPA[4], A. KEPECS[2], B. ROZSA[1]; [1]IEM-HAS, Budapest, Hungary; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [3]Pázmány Péter Catholic Univ., Budapest, Hungary; [4]Femtonics Ltd., Budapest, Hungary

Abstract: Vasoactive intestinal polypeptide (VIP) demarcates a small, unique population of inhibitory interneurons, making up 1-2% of all neocortical neurons. VIP interneurons are mainly located in the superficial layers of the neocortex and provide inhibition onto other inhibitory interneurons, most SOM and a fraction of PV neurons, thus disinhibiting principal cells. While the circuit impact of VIP interneurons has been well characterized, less is understood about their behavioral function. Here we set out to examine how VIP interneurons respond during simple choice behaviors. Measuring of sparse interneuron populations as the VIPs, is a challenging imaging task. Our aim was to record activity of over 100 interneurons simultaneously, while mice are behaving. We developed an improved 2P 3D acousto-optic microscope, and new scanning methods capable of measuring hundreds of neuronal somata. As a result, we measured simultaneously the activity of Gcamp6f - labeled VIP neurons in a 450 μm x 450 μm x 650 μm large volume. The half-cubic-millimeter scan range, with a high scanning speed (up to 500 pts per kHz), with less than 400 nm resolution in the center core and less than 1.9 μm × 1.9 μm × 7.9

μm resolution throughout the entire scanning volume allows us a very precise functional mapping at different areas and depths of the mouse neocortex. We also developed a high-precision motion artifact compensation algorithm to make possible valid measurement of small neural tissue volumes during activity. Here we present the results, analysis and interpretation of this series of measurements along with the motion-compensation algorithm and its possible uses in in vivo experiments with behaving animals.

Disclosures: **Z. Szadai:** None. **H. Pi:** None. **G. Katona:** None. **T. Tompa:** None. **A. Kepecs:** None. **B. Rozsa:** None.

Poster

560. Optical Methods: Real-Time Imaging

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Program#/Poster#: 560.02/NNN20

Topic: I.04. Physiological Methods

Support: Simons Foundation

Swartz Foundation

Pew Trust

Klingenstein-Simons Fellowship

Title: Cellular-level resolution of barrel cortex borders using calcium imaging

Authors: ***M. T. KAUFMAN**, S. MUSALL, A. K. CHURCHLAND;
Neurosci., Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Brain areas are traditionally defined posthumously by cytoarchitecture, histological markers, or neural projection patterns. Our ability to identify areas in vivo is more limited. One common technique is wide-field functional imaging, which relies on repeated presentation of sensory stimuli and averaging across neural responses. However, this approach provides limited spatial resolution and is only viable for areas with clear stimulus tuning. To address this issue, we used two-photon calcium imaging in awake mice to detect column borders at cellular resolution based on neural correlation patterns. We imaged the spontaneous activity of ~1,000 neurons at a time in barrel cortex of GCaMP6f transgenic animals. Neurons were identified automatically, using a published variant of constrained non-negative matrix factorization. We then examined the correlation patterns between neurons' activity using low-dimensional embedding techniques or formal clustering methods (inspired by Kiani et al., 2015 Neuron). In

many datasets, this approach revealed clearly identifiable barrel borders that were consistent across several imaging depths and repeated imaging over days. When visible, borders of individual barrels were mostly clear-cut with little intermingling of cells or obvious gradients at the transitions. The locations of individual barrels were also consistent with results from wide-field imaging when stimulating individual whiskers. As expected, the correlations between neurons also tended to smoothly decrease with distance. The strength of correlation with distance was often well fitted by a double-exponential distribution. Both the exponential falloff and individual barrel structure were much weaker at superficial imaging planes less than 150 um deep. In summary, our results demonstrate that noise correlations can be used to identify column borders at cellular resolution in vivo. Our method does not require specific stimuli to assess correlation between neurons and might therefore be particularly useful to identify higher-order brain structures with more complex tuning properties. Further, our classification of single neurons provides insight into the sharpness of functional borders, which is of particular interest when studying borders between different brain areas.

Disclosures: **M.T. Kaufman:** None. **S. Musall:** None. **A.K. Churchland:** None.

Poster

560. Optical Methods: Real-Time Imaging

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Topic: I.04. Physiological Methods

Support: NIH Grant 5R01MH101198

Simons Foundation Circuits Grant

Title: Cortex-wide cellular-resolution imaging with ultra-large cranial window reveals regional differences in state-dependent modulation in behaving mice

Authors: *B. S. HUANG, A. BELLAFARD, S. VRONTOU, P. GOLSHANI;
Dept of Neurol., UCLA Sch. of Med., Los Angeles, CA

Abstract: Behavioral states, such as locomotion and startle, have been shown to increase cortical neuronal excitability and network activity, in a process involving cortex-wide release of neuromodulators (e.g. norepinephrine) from subcortical nuclei such as the locus coeruleus. However, it is unclear whether these state-dependent modulations exert a homogeneous global effect on the entire cortex, or regional differences in circuit architecture could produce heterogeneous local responses. Two-photon calcium imaging in head-fixed behaving mice has

enabled studies of state-dependent circuit dynamics at cellular resolution, but one current limitation is the need to restrict imaging to a pre-selected region-of-interest. This targeted approach may obscure potential regional differences in state-dependent modulations. To overcome such limitation, we developed a cortex-spanning cranial-window preparation that provides long-term cellular-resolution access to the entire dorsal cortical surface of adult mice. We also incorporated right-angle microprisms within our ultra-large window to image regions such as the mPFC (medial prefrontal cortex), ACC (anterior cingulate cortex), and RSCm (medial retrosplenial cortex) that are buried within the midline fissure. To study state-dependent modulations, we implemented a simple startle paradigm to head-fixed mice that were free to run and rest on a spherical treadmill. Using transgenic GCaMP6-expressing mice, we imaged calcium activity in layer-2/3 pyramidal neurons across the cortex along the anterior-posterior axis. We analyzed neuronal activity during spontaneous vs. startle-induced locomotion and found distinct regional differences to these state-dependent modulations within individual animals.

Disclosures: **B.S. Huang:** None. **A. Bellafard:** None. **S. Vrontou:** None. **P. Golshani:** None.

Poster

560. Optical Methods: Real-Time Imaging

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Support: NIH 5T32NS58280

Title: Calcium imaging of hippocampal cell activity in behaving rats

Authors: *G. BLAIR, A. G. HOWE, D. AHARONI, S. FLORES, T. SHUMAN, P. GOLSHANI, H. T. BLAIR;
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Abstract: We have used the UCLA miniscope (miniscope.org) to image calcium dependent fluorescence activity bilaterally in the rat hippocampus during open foraging behavior. Rats were infused with a GCamp6f AAV virus (0.6 ul per hemisphere) directly in the CA1 pyramidal cell layer. A week later, GRIN lenses (5 mm length x 2 mm diameter) were implanted on the dorsal surface of the hippocampus centered at coordinates -3.6 posterior and +/- 2.9 lateral to bregma, after vacuum aspiration of the overlying cortex. The white matter of the corpus callosum was also removed, but the white matter of the alveolus overlying the hippocampus was left intact. Lenses were lowered to make gentle contact with the surface of the alveolus, then secured in

place with a combination of cyanoacrylate glue and bone cement. Ten days after lens implantation, a base plate (for holding the miniscope) was mounted over the GRIN lens at an angle optimized for viewing the field of fluorescent neurons. After recovery, real time imaging was carried out with the miniscope as rats foraged for 20 mg food pellets in an open field. Using these methods, we have successfully measured activity-dependent fluorescence changes in hippocampal neurons, some of which exhibit spatially selective firing. It remains unclear whether these neurons are pyramidal cells in the CA1 layer, because the pyramidal layer lies at a depth of 300-400 um below the lens surface, which is near the limit of the effective imaging depth for the lens. Further studies are in progress to determine whether the hippocampal pyramidal layer lies within the range of imaging depth using this approach.

Disclosures: **G. Blair:** None. **A.G. Howe:** None. **D. Aharoni:** None. **S. Flores:** None. **T. Shuman:** None. **P. Golshani:** None. **H.T. Blair:** None.

Poster

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Title: *In vivo* image of DRG neurons: a new way to investigate pain pathways.

Authors: ***A. P. LUIZ**[1], E. C. EMERY[1], Q. MILLET[1], X. DONG[2], J. N. WOOD[1]; [1]Dept. of Med., WIBR/University Col. London, London, United Kingdom; [2]Sch. of Med., Howard Hughes Med. Institute/Johns Hopkins Univ., Baltomire, MD

Abstract: New approaches to investigate pain mechanisms could facilitate our understanding of different pain pathways. Here we describe a novel method for studying DRG neuronal function, *in vivo*, using GCaMP imaging. Mice expressing GCaMP3 under the DRG neuron-specific

promoter Pirt were used to investigate DRG physiology *in vivo*. Pirt-GCaMP3 mice (8-10 weeks) were anaesthetised (ketamine/medetomidine: 120/1.2 mg/kg) and the L2-L5 section of the vertebral column was exposed. Transverse and superior articular processes of the vertebra were removed in order to visualise the DRG. Once exposed, the animal was fitted to a custom spinal clamp and fixed to a single-photon confocal microscope (Leica SP8). The trunk of the animal was slightly raised to reduce respiratory noise. Once the animal was stable, various tactile and noxious stimuli were applied to the ipsilateral rear paw and any associated changes in neuronal activity were recorded as a function of GCaMP fluorescence. To explore the utility of the model, *in vivo* imaging was performed on mice lacking the sodium channel $Na_V1.8$. DRG from $Na_V1.8^{-/-}$ mice showed reduced responses to mechanical stimulation compared to WT mice, consistent with reported behavioural data. Interestingly, there was no difference in response to cold stimulation (0^oC), which was supported by acetone and cold plate behavioural assays. Overall, these results present a novel method for studying DRG neuron function *in vivo*, which can be used to further investigate discrete pain pathways.

Disclosures: **A.P. Luiz:** None. **E.C. Emery:** None. **Q. Millet:** None. **X. Dong:** None. **J.N. Wood:** None.

Poster

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HHMI

NSF

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the U.S. Army Research Laboratory and Defense Advanced Research Projects Agency (Cooperative Agreement Number W911NF-14-2-0013)

Title: Activity-dependent CLARITY whole brain analysis reveals long-range wiring and molecular signatures of prefrontal ensembles mediating positive or negative valence experience

Authors: *L. YE[1,2,3], W. ALLEN[1,4], K. THOMPSON[1], Q. TIAN[5], B. HSUEH[1], C. RAMAKRISHNAN[1], A.-C. WANG[1], J. JENNINGS[1], A. ADHIKARI[1], C. HALPERN[6], I. WITTEN[1], A. L. BARTH[7], L. LUO[2,4], J. A. MCNAB[5], K. DEISSEROTH[1,2,3];
[1]Bioengineering, [2]Howard Hughes Med. Inst., [3]Psychiatry & Behavioral Sci., [4]Biol., [5]Radiology, [6]Neurosurg., Stanford Univ., Stanford, CA; [7]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: A major challenge in understanding the cellular diversity of the brain has been linking activity during behavior with standard cellular typology. For example, it has not been possible to determine if the principal neurons in prefrontal cortex that are differentially active during distinct experiences represent separable cell types (based on anatomy and molecular properties), nor is it known if these differentially-active cells actually exert distinct causal influences on behavior. Here we report development of fast and scalable CLARITY technologies, tailored for large-scale behavioral cohorts, to enable automatic intact-brain cellular resolution activity mapping and quantitative brainwide axonal projection analysis. Combined with transgenic activity labeling (ArcTRAP) or single-component activity-dependent virus (c-fos-CreER or -ChR2 AAVs) strategies, this system allows us to connect neuronal activity in cells reporting on behavioral experience with measures for both brainwide wiring and molecular phenotype of the same cells. We first performed whole brain activity mapping using ArcTRAP mice labelled with positive (cocaine administration) or negative (foot shock) valence and found that both experiences recruited activity in the mPFC (2.6 and 1.8 fold increase in TRAP cell numbers in cocaine and shock groups, respectively, compared to home cage, n=5 per group, $p<0.05$). Importantly, these experiences in mPFC are represented by cell populations that are similarly positioned, but differ in causal impact on behavior (as determined by activity-dependent optogenetics and real-time place preference, n=10-14 per group, mean preference change for cocaine: 1.3x +/- 0.1, Wilcoxon p=0.0006; for shock: 0.8x +/- 0.1, Wilcoxon p=0.002), long-range wiring (i.e., cocaine-activated population preferentially projects to the NAc compared to shock-activated population, as determined by CLARITY projection analysis, n=6 per group, $p<0.05$), and gene expression profile—with the major molecular discriminant being expression of the adaptation-linked transcription factor NPAS4. These findings illuminate the cellular logic of mPFC information processing and adaptive behavior, and may point the way to cell type-specific understanding and treatment of disease-associated states.

Disclosures: L. Ye: None. **W. Allen:** None. **K. Thompson:** None. **Q. Tian:** None. **B. Hsueh:** None. **C. Ramakrishnan:** None. **A. Wang:** None. **J. Jennings:** None. **A. Adhikari:** None. **C. Halpern:** None. **I. Witten:** None. **A.L. Barth:** None. **L. Luo:** None. **J.A. McNab:** None. **K. Deisseroth:** None.

Poster

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Title: Probing the brain-wide neural dynamics of passive coping behavior

Authors: *A. S. ANDALMAN[1], V. M. BURNS[2], M. BROXTON[3], B. POOLE[4], S. J. YANG[5], L. GROSENICK[4,8], M. LOVETT-BARRON[4], T. N. LERNER[4], N. PICHAMOORTHY[4], P. MOURRAIN[6], M. LEVOY[3,9], K. DEISSEROTH[6,10,7];
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Abstract: Hopelessness is a diagnostic criterion for major depression and can manifest as a *maladaptive* discounting of the value of effort in the present. Yet, in the context of an inescapable adverse environment, similar discounting can be *adaptive* as a means to conserve energy and minimize risk, and is a commonly observed behavioral response across species. In rodents, both the forced swim test and the tail suspension test cause a transition to passive coping in which minimal energy is expended. The transition to passive coping in rodents is therefore not only a validated assay for clinical antidepressant effects, but also a conserved behavioral state transition of substantial adaptive value and basic-science interest.
To advance understanding of the neural dynamics associated with passive-coping states, we developed a protocol to induce passivity in the larval zebrafish (13-16 dpf). With exposure to inescapable shocks, we found that the fish enter a passive coping-like state in which movement is

reduced compared to controls (n=24; -1.78 mm/s, $p<0.001$). We found that as in rodents, the onset of this state can be delayed by prior exposure to the acute experimental antidepressant ketamine (n=16; 6 min, $p<0.001$). To measure neural dynamics associated with this passive coping state, we used light field microscopy to acquire synchronous whole-brain measurements of neural activity in fish expressing a genetically encoded Ca^{2+} indicator pan-neuronally. We observed that our protocol causes a pronounced suppression of brain-wide neural activity (n=5; 83% of control, $p <0.005$). The time-course of this suppression was similar to the time-course of the behavioral effects, and prior exposure to ketamine was found to delay the suppression. Finally we tested whether this experience could have lasting effects on future responses to aversive stimuli. We tested this by examining behavioral and neural responses to intermittent probe shocks in fish three hours after exposure to the shock protocol. We found that the average speed of naïve fish was unaffected, but previously-exposed fish exhibited reduced speed (n=13; -0.6 mm/s, $p<0.01$), revealing stable behavioral plasticity consistent with increased passivity in response to aversive stimuli. In addition, calcium imaging revealed that neurons in the ventral/lateral habenula (only in previously-exposed fish) exhibited slowly-ramping increases in neural activity in response to the intermittent aversive stimuli (n=10; 272% of control, $p<0.05$). Together, these experiments provide brain-wide, cellular-resolution and real-time insight into the mechanisms of both immediate and long-lasting passive-coping responses.

Disclosures: **A.S. Andalman:** None. **V.M. Burns:** None. **M. Broxton:** None. **B. Poole:** None. **S.J. Yang:** None. **L. Grosenick:** None. **M. Lovett-Barron:** None. **T.N. Lerner:** None. **N. Pichamoorthy:** None. **P. Mourrain:** None. **M. Levoy:** A. Employment/Salary (full or part-time): Google. **K. Deisseroth:** None.

Poster

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Support: NIH Grant R01 DA07418

Title: Piezo-assisted cranial surgery: a novel technique for improving cranial window surgeries in rodents.

Authors: *J. M. LAWSON, R. M. MIKOFSKY, S. CLARK, D. SULZER;
Sulzer Lab. - Neurol., Columbia Univ., New York, NY

Abstract: Piezosurgery is a relatively new technique that utilizes high frequency vibrations to remove bone without damaging soft tissue. This has been extensively applied to maxillofacial and dental surgery in humans. This technique, however, has not been applied to creating chronic optical windows for multiphoton imaging in mice. Multiphoton microscopy is a widespread technique used to image neurons *in vivo*. The two main current techniques to prepare the bone for chronic imaging are cranial window surgery and thin skull preparations. Both of these techniques utilize a high speed drill that can increase the risk of damaging the brain tissue. These traditional cranial surgical techniques can be difficult to perform, time consuming, and require extensive training. We have developed a novel method for applying piezosurgery (Mectron) to optimize cranial window preparations for improved multiphoton imaging in mice. Overall, our method is easier to learn, has a greater average success rate, and allows for higher precision over traditional cranial window surgery. Furthermore, this technique allows for shorter surgeries, minimizing surgery-related complications with the animal. Piezo-assisted cranial surgery has the potential to expand the use of multiphoton imaging *in* vivo by improving outcomes of cranial window surgery in rodents and allowing for more extensive imaging in previously understudied brain regions.

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Poster

560. Optical Methods: Real-Time Imaging

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Topic: I.04. Physiological Methods

Title: Fast 3D functional imaging of neuronal networks & dendritic spine assemblies in behaving animals

Authors: *S.-J. LINDA[1], G. SZALAY[1], Z. SZADAI[1], K. ÓCSAI[2], M. VERESS[3], B. CHIOVINI[1], P. MAÁK[3], G. KATONA[2], B. RÓZSA[1];
[1]Inst. of Exptl. Med. of the Hungaria, Budapest, Hungary; [2]MTA-PPKE ITK-NAP B – 2p measurement technology group, The Fac. of Information Technology, Pázmány Péter Catholic Univ., Budapest, Hungary; [3]Dept. of Atomic Physics, Budapest Univ. of Technol. and Econ., Budapest, Hungary

Abstract: Understanding brain function requires novel imaging methods such as 3D random-access point scanning that can simultaneously read out neural activity on both the dendritic and somatic scales. Our 3D AO scanning method can increase measurement speed and signal-to-noise ratio by up to 6-9 orders of magnitude, but suffers from one main disadvantage:

fluorescence information is lost during brain movement in awake, behaving animals as the amplitude of brain motion is much larger than the diameter of a single excitation spot. In this work we present a novel fluorescent imaging technology, 3D drift AO scanning microscopy, which can extend each scanning point to small 3D lines or surface or volume elements, preserving fluorescence information for fast off-line motion correction. Our method effectively eliminates *in vivo* motion artifacts, allowing fast 3D measurement of over 100 dendritic spines with 3D lines, over 100 somata with squares and cubes, or multiple spiny dendritic segments with surface and volume elements in moving animals. Finally, a four-fold improvement in total excitation efficiency resulted in a large, about 500µm × 500µm × 650µm, scanning volume with genetically encoded sensors.
We used the new technology to record activity of inhibitory neurons in the moving brain of behaving animals. We revealed a new, broadcasted signaling pathway which activates learning mechanism through the entire neocortex during reward and punishment.

Disclosures: **S. Linda:** None. **G. Szalay:** None. **Z. Szadai:** None. **K. Ócsai:** None. **M. Veress:** None. **B. Chiovini:** None. **P. Maák:** None. **G. Katona:** None. **B. Rózsa:** None.

Poster

560. Optical Methods: Real-Time Imaging

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Title: Temporal precision of deconvolution when imaging slow Ca^{2+} indicators

Authors: *J. B. ISSA, M. BEN-JOHNY, D. T. YUE;
Johns Hopkins Univ., Baltimore, MD

Abstract: Calcium indicators represent the dominant fluorescence reporter of neuronal activity as they afford superior signal-to-noise and correlate reliably with electrical activity. A major limitation, however, is the temporal resolution of Ca^{2+} indicators. Whereas transmembrane voltage during a neuronal action potential is fast, on the order of one millisecond, associated Ca^{2+} signals are much slower, lasting tens of milliseconds. Compounding this problem are the slow binding kinetics of Ca^{2+} indicators, especially genetically encoded Ca^{2+} indicators (GECIs). A potential solution is to deconvolve the fluorescence signal using a kernel that describes the

impulse response of the Ca^{2+} indicator, yielding an estimate of the underlying spike train of the neuron. One widely used method, termed fast non-negative deconvolution, assumes a single-exponential decay as the kernel. However, for many GECIs, the on-kinetics of binding cannot be assumed to be instantaneous, especially under fast imaging. Here we ask whether the biophysically-realistic properties of GECIs can be simplified for use in a computationally fast deconvolution strategy and how well such an algorithm performs on both simulated and real data. First, we formulate a biophysical model that explains the temporal dynamics of Ca^{2+} indicators. Second, we develop an optimal deconvolution algorithm, which we test on simulated data to determine the maximal amount of temporal resolution that could be observed under experimental conditions. Third, we test our model and assumptions on publicly available *in vitro* data where Ca^{2+} imaging and whole cell patch clamping were performed simultaneously. Finally, we test our model on *in vivo* data from the mouse auditory cortex. We find that, while slow binding kinetics do limit the temporal resolution of spike inference from deconvolution, precision on the order of milliseconds can be achieved even with 'slow' indicators such as GCaMP6s, thus opening the door for studies on temporal encoding when utilizing Ca^{2+} imaging.

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Poster

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Support: NSF Award No. 1541612

Title: Alterations in sensory-activated cerebral blood flow following brain injury in mice

Authors: *H. JANG[1,2], M. YE[2], S. HUANG[2], D. X. HAMMER[2], C. G. WELLE[2,3], J. A. N. FISHER[1,2];
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Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability in persons under the age of 45 years. Symptoms of brain injury might not appear until days or weeks following the incident and may go unnoticed as patients may appear to have recovered, despite acting or feeling differently. However, quantitative "gold standard" biomarkers for diagnosing TBI, particularly mild TBI (mTBI), are lacking. Clinical imaging modalities, namely magnetic

resonance imaging (MRI) and computed tomographic (CT) imaging, provide quantitative and anatomically specific data, however changes due to mTBI can be more difficult to detect with standard imaging protocols. Recent work has demonstrated that sensory evoked electroencephalographic (EEG) potentials can be utilized to detect and monitor acute brain injury (Fisher et al., *IEEE Trans. Neural Sys. Rehab.* 2016). Evoked hemodynamic and metabolic responses to the same somatosensory stimuli, which have been recorded using non-invasive near infrared (NIR) optical techniques, are also potential biomarkers. Although work on the diagnostic potential of EEG and on NIR optical techniques have been largely in isolation of each other, the two techniques measure signals—electrical activity and the ensuing hemodynamic and metabolic response—that jointly report the function of the "neurovascular unit," a fundamental dynamic coupling between individual neurons and nearby microvasculature that comprises electrophysiological interaction, mechanical connectivity, chemical signaling, among many other factors. We sought to explore whether a correlate of these injury-induced alterations in sensory activation could be detected at the level of local cerebral blood flow (CBF). Diffuse correlation spectroscopy (DCS) is an emerging portable diffuse NIR modality that has been used for measurements of CBF (Durduran & Yodh, *NeuroImage* 2014). Among other optical techniques, DCS has the unique advantage of being sensitive primarily to CBF in microvasculature. We explored potential sensory-activated CBF biomarkers *in vivo* by performing continuous DCS measurements during somatosensory stimulation of the median nerve in mice following controlled cortical impact (CCI). A 3D-printed miniaturized probe that housed micro-prisms permitted stable fixture of light-emitting source and detector-coupled fibers against the skull region overlying the somatosensory cortex. Our preliminary results demonstrate in mice that somatosensory-evoked CBF detected optically can potentially serve as useful diagnostic biomarkers for rapid detection of sublethal brain injury.

Disclosures: H. Jang: None. **M. Ye:** None. **S. Huang:** None. **D.X. Hammer:** None. **C.G. Welle:** None. **J.A.N. Fisher:** None.

Poster

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Title: Propagating network activity assessed in a tangential cortical slice

Authors: *C. C. LEE;
Comparative Biomed. Sci., Louisiana State Univ. Sch. of Vet. Med., Baton Rouge, LA

Abstract: The propagation of activity in the cerebral cortex relies on both local and long-range network connections. In this manner, information is communicated among different cortical stations to integrate incoming sensory streams with ongoing network activity. The propagation of such activity across the cerebral cortex relies on both the network architecture and the balance of excitatory and inhibitory inputs. To approach the characterization of propagating cortical activity, we employed an in vitro tangential slice preparation of the sensory neocortex in the mouse. We utilized flavoprotein autofluorescence to measure the spatial and temporal activity following network perturbation. In addition, we employed laser-scanning uncaging via glutamate uncaging to probe functional connectivity in these tangential slices. We found widespread but variable flavoprotein activity in our tangential slices, but with limited patterns of convergent inputs to single neurons. Our results suggest that in this preparation, local connectivity may be sufficient to propagate activity across the cortical surface and influence computational processes on a global scale.

Disclosures: C.C. Lee: None.

Poster

560. Optical Methods: Real-Time Imaging

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Topic: I.04. Physiological Methods

Support: NEI R01s EY022129

NEI R01s EY026766

Title: Application of gradient index lens for *In vivo* fluorescent microscopy of retinal ganglion cells

Authors: *D. BUICKIANS[1], A. KREYMERMAN[2], E. HUYNH[1], Y. GONG[1], H. CHEN[1], J. L. GOLDBERG[2];
[1]Bioengineering, UCSD, La Jolla, CA; [2]Ophthalmology, Stanford, Palo Alto, CA

Abstract: Subcellular pathological changes that manifest as a consequence of disease or injury within the central nervous system are often captured post-mortem using conventional or electron microscopy. This has fundamentally limited our understanding of neuronal processes that precede or progress during neuronal dysfunction and degeneration. Recent biomedical imaging technology such as 2-photon or confocal microscopy, has proven to be effective in capturing images of CNS disease related changes, with high magnification and resolution. Using these imaging techniques, events such as mitochondrial transport and fission/fusion deficits, axonal swelling, and eventual axon fragmentation/breakdown have been proven as hallmarks for many neurodegenerative diseases or traumas. However, due to physical accessibility, invasive procedures, and/or the low resolution of externally applied imaging technology (i.e. optical coherence tomography, heidelberg confocal scanning laser ophthalmoscopy MRI), sites within the CNS such as the retina, optic nerve and various deep brain regions, have proven to be more difficult to image. Consequently, cellular level in vivo microscopy (IVM) has been limited to mostly superficial structures, leaving many of the CNS regions susceptible to degenerative diseases or injury unstudied. To ovefrcome the current limitations in cellular level IVM, we have been adapting common 2-photon or confocal microscopy with gradient index (GRIN) optical lens. GRIN lens have small diameters and moderate lengths, while maintaining relatively high numerical apertures. Here we present our current design and preliminary evidence for the application of grin lens for in vivo imaging of the optic nerve and retina. Here we show that, coupled with minimally invasive surgery, the physical properties of GRIN lens confer imaging properties difficult to reach CNS structures with high spatio-temporal resolution. Future studies will focus on imaging of the peri/intra-axonal/dendrite changes that accompany retinal ganglion cell degeneration in rodent optic neuropathy modeling.

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Poster

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Topic: I.04. Physiological Methods

Title: Spectral domain optical coherence tomography for the assessment of cerebrovascular plasticity

Authors: *J. J. KAY[1], F. ATRY[2], A. T. WICKSTROM[1], J. R. KRUEGER[1], R. PASHSAIE[2], R. A. SWAIN[1];
[2]Electrical Engin., [1]Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Vascular pathologies represent the leading causes of mortality worldwide. Cerebral hypoxia is a condition that often manifests as a result of these medical conditions. Remarkably, the nervous system has evolved mechanisms to compensate for oxygen deprivation. For example, the dilation of existing vessels and the growth of new vessels are two prominent physiological processes that play a critical role in the maintenance of cerebral homeostasis. Recently, exercise has been shown to induce a mild state of hypoxia in the brain, leading to several robust morphological changes within the cerebrovascular system. Thus, exercise serves as a viable model for investigating hypoxia-induced adaptations. The present study introduces spectral domain optical coherence tomography (SD-OCT) as a novel technique for examining these micro-level changes in the rat motor cortex. SD-OCT produces high resolution, 3D angiograms, and allows for moderately invasive imaging within the same animal at multiple time points. The independent effect of exercise training on cerebrovascular structure and function has never been explored using SD-OCT. Thus, the primary goal of this study was to determine the relative efficacy of SD-OCT utility. To validate SD-OCT, we employed it in the examination of exercise-dependent changes in blood vessel density and real-time capillary dilation during a laboratory-induced condition of hypoxia. In addition, histology data was collected to provide comparative measures for statistical analyses. At the start of this investigation, animals were randomly assigned to one of two groups: 26-week voluntary exercise (VX), or an inactive control (IC). Upon completing the exercise treatment, animals were anesthetized and prepared for imaging. Vascular anatomy and blood velocity data was captured during three experimental conditions: [1] normal oxygen baseline, [2] hypoxia, and [3] normoxia. A two-way analysis of variance revealed a significant difference in total blood vessel density between treatment groups, independent of condition. That is, VX animals had a greater density of blood vessels in the scanned region of interest when compared to IC. These findings were confirmed using unbiased stereology techniques to analyze tissue in the scanned region of interest. Furthermore, statistical analyses revealed a significant increase in small arteriole diameter in both VX and IC animals. However, the dilation captured by SD-OCT was significantly greater in VX animals when compared to IC. In sum, exercise induces potent adaptations that promote greater flexibility during hypoxia. Moreover, these micro-level changes can be effectively probed using SD-OCT.

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Poster

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CEITEC 2020 LQ1601

Title: Alterations in cerebral metabolism observed *In vivo* using fluorescence lifetime microscopy of intrinsic NADH

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Abstract: Monitoring cerebral energy metabolism at a cellular level is essential for guiding our understanding of healthy brain function and its pathological alterations. In this study, we resolve specific alterations in cerebral metabolism utilizing minimally-invasive 2-Photon fluorescence lifetime imaging (2P-FLIM) measurements of reduced nicotinamide adenine dinucleotide (NADH) fluorescence, collected in vivo from the exposed cortices of anesthetized rats and mice. Time-resolved lifetime measurements enables distinction of different components contributing to NADH autofluorescence. These components reportedly represent different enzyme-bound formulations of NADH. Our observations from this study confirm the much-repeated hypothesis that NADH FLIM can identify specific alterations in cerebral metabolism. Using time-correlated single photon counting (TCSPC) equipment and a custom-built multimodal imaging system, 2-photon fluorescence lifetime imaging (FLIM) was performed in cerebral tissue with high spatial and temporal resolution. Animals were anesthetized, mechanically-ventilated, and their cerebral cortices were exposed through a sealed cranial window. Multi-exponential fits for NADH fluorescence lifetimes indicate 4 distinct components, or 'species.' We observed distinct

variations in the relative proportions of these components before and after pharmacological-induced impairments to several reactions involved in anaerobic glycolysis and aerobic oxidative metabolism. Classification models developed with experimental data correctly predict the metabolic impairments associated with bicuculline-induced focal seizures in separate experiments. Compared to traditional intensity-based NADH measurements, lifetime imaging of NADH is less susceptible to the adverse effects of overlying blood vessels. Evaluating NADH measurements will ultimately lead to a deeper understanding of cerebral energetics and its pathology-related alterations. Such knowledge will likely aid development of therapeutic strategies for neurodegenerative diseases such as Alzheimer's Disease, Parkinson's disease, and stroke.

Disclosures: **M.A. Yaseen:** None. **S. Sakadžić:** None. **J. Sutin:** None. **W. Wu:** None. **B. Fu:** None. **H. Uhlirova:** None. **A. Devor:** None. **D.A. Boas:** None.

Poster

560. Optical Methods: Real-Time Imaging

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Topic: I.04. Physiological Methods

Support: NIH R24 Grant NS092986

Title: Facilitating the adoption of oxygen partial pressure imaging with two-photon microscopy

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Abstract: The assessment of brain oxygenation on the microscopic level has the potential to transform our understanding of important clinical problems, such as stroke, Alzheimer's disease, dementia, chronic hypertension, and brain cancer, facilitating the development of new therapies and helping to improve clinical imaging and treatment protocols. Until now, no technology has been capable of microscopic oxygen imaging in the brain with high spatial and temporal resolution. Over the past several years we have developed a method, termed two-photon phosphorescence lifetime microscopy of oxygen (2PLM), which has the unique capability of fulfilling this niche. This is the only imaging method that allows high resolution mapping of brain oxygenation in real time.
2PLM of oxygen is a combination of state-of-the-art two-photon enhanced phosphorescent

probes and a unique variant of two-photon laser scanning microscopy - both of which are not presently available commercially. The transformative power of 2PLM of oxygen has been demonstrated in several high-impact publications [1-7], producing great interest in the neuroscience community. With the help of the NIH R24 grant mechanism, we are setting up a self-sustaining resource that will promote widespread use of the two-photon oxygen imaging technology, making this new powerful method available to a broad group of neuroscience researchers.

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Disclosures: S. Sakadzic: None. **T.V. Esipova:** None. **M.A. Yaseen:** None. **A. Devor:** None. **S.A. Vinogradov:** None. **D.A. Boas:** None.

Poster

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Support: NIH grant NS091230

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Title: Two-photon microscopy measurement of cerebral metabolic rate of oxygen using periarteriolar oxygen concentration gradients

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Abstract: *Objective.* The cerebral metabolic rate of oxygen ($CMRO_2$) is an essential parameter for evaluating brain function and pathophysiology. Measurements of $CMRO_2$ with high spatio-temporal resolution are critically important for understanding how the brain copes with metabolic and blood perfusion changes associated with various clinical conditions, such as stroke, periinfarct depolarizations, and various microvasculopathies (e.g., Alzheimer's disease, chronic hypertension). $CMRO_2$ measurements are also important for understanding the physiological underpinnings of functional Magnetic Resonance Imaging signals. However, the currently available approaches for quantifying $CMRO_2$ rely on complex multimodal imaging and mathematical modeling. Here, we introduce a novel method that allows estimation of $CMRO_2$ based on a single measurement modality - two-photon phosphorescence lifetime microscopy (2PLM) imaging of the partial pressure of oxygen (PO_2) in cortical tissue.
Methods. We measured the baseline $CMRO_2$ in anesthetized rats, and modulated tissue PO_2 levels by manipulating the depth of anesthesia. $CMRO_2$ is estimated by fitting the changes of tissue PO_2 around cortical penetrating arterioles with the Krogh cylinder model of oxygen diffusion.
Results. Using this method, we obtained a mean baseline $CMRO_2$ of 1.71 ± 0.16 μmol cm^{-3} min^{-1}, within the error bounds of previously reported $CMRO_2$ under similar anesthesia in rats measured by MRI (2.5 ± 1.0 μmol cm^{-3} min^{-1}) [1]. To experimentally manipulate $CMRO_2$, we modulated the level of anesthesia by applying isoflurane (2%) on top of the ongoing alpha-chloralose anesthesia. Adding isoflurane resulted in the measured $CMRO_2$ decreased from 1.56 ± 0.07 μmol cm^{-3} min^{-1} (alpha-chloralose only) to 1.38 ± 0.07 μmol cm^{-3} min^{-1} (combined alpha-chloralose and isoflurane).
Conclusion. Our study demonstrates that we can estimate $CMRO_2$ using the Krogh cylinder model based on a single measurement modality - periarteriolar tissue PO_2 measurement by two-photon microscopy in a single plane perpendicular to the vessel axis. With this method, no measurements of blood flow are required for the $CMRO_2$ estimation. This method has a spatial resolution of approximately 200 μm and it may provide $CMRO_2$ measurements in individual cortical layers or within confined cortical regions such as in ischemic penumbra and the foci of functional activation.

[1] P. Hermán, H. K. F. Trübel, and F. Hyder, “A multiparametric assessment of oxygen efflux from the brain,” J. Cereb. Blood Flow Metab. 26(1), 79-91 (2005).

Disclosures: **S. Sakadzic:** None. **M.A. Yaseen:** None. **R.S. Jaswal:** None. **E. Roussakis:** None. **A.M. Dale:** None. **R.B. Buxton:** None. **S.A. Vinogradov:** None. **D.A. Boas:** None. **A. Devor:** None.

Poster

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Topic: I.04. Physiological Methods

Support: National Natural Science Foundation of China under Grant No. 2030001130013

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Title: Real-time monitoring of the brain interstitial fluid flow in tumor-bear rats using the tracer-based MRI

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Abstract: Objective: Study the diffusion and flow parameters in the brain extracellular space at the different stage of glioma in the C6 modeling rats’ brains using the tracer-based MRI. Methods: 16 adult male SD rats bearing glioma which were implanted C6 cells in the striatum were randomly divided into two groups. Both were examined using the tracer-based MRI and routine MR imaging, with one group bearing the glioma in 10 days, and the other group were 20 days. The flow and diffusion parameters of the brain interstitial space were examined, and the dynamic distribution and the clearance of the probe molecules were continuously monitor in Real-time using MRI. The parameters were compared between two groups. Results: The tumor size were increased, but the maximum volume of distribution (Vd max) in the brain interstitial space were not statistically different in the two groups. The clearance of the probe in 10 days group was faster than that in 20 days group with a shorter half time of (0.86±0.23h vs. 1.64±0.12h, $t=5.91$; $p<0.001$) hours. Conclusion: The diffusion and flow properties within the tumor are both ruined as glioma grows, which can be detected using tracer-based MRI.

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Poster

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Topic: I.04. Physiological Methods

Support: KHIDI grant HT15C0001

Title: Optical coherence elastogram of mouse cerebral cortex

Authors: Y. CHANG[1], J. KIM[2], J. HWANG[2], Y.-S. OH[3], *C. SONG[4,1];
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Abstract: Mechanical properties of the brain tissue including its elasticity may be altered during the progress of various neurological disorders including Alzheimer disease, hydrocephalus, neuroinflammation, and stroke. To detect subtle changes in the elasticity of the soft brain tissue, advanced modality is required for high resolution assessment. Here, we present an optical coherence elastographic (OCE) system capable of measuring the elasticity of cerebral cortex. This approach achieves more accurate measurement rather than magnetic resonance elastography or ultrasonic elastographic imaging.
The proposed OCE system capable of measuring the elasticity is based on fiber optic spectral domain optical coherence tomography (SD-OCT). A broadband laser source with a center wavelength of 830 nm is used. Spectral interference signals are acquired by a line CMOS camera with 4096 pixels in a home-built spectrometer. A non-contact immersion type transducer is used to generate short duration acoustic radiation force to the cerebral cortex. The displacements of the cerebral cortex are measured while acoustic radiation force is applied to the cerebral cortex. The dissected mouse cortex tissue was mounted on the plastic plate filled with saline solution. The displacements of various sub-regions in the cortex are measured. The measured displacements are used to calculate Young's modulus which indicates the stiffness throughout the cerebral cortex.
The OCE system allowed to measure the elasticity of sub-regions in the mouse cerebral cortex. Mapping any elastic change in the CNS with the OCE system may open up an unprecedented window to monitor the physiological abnormality in the brain. To map biomechanical properties of the brain may contribute to our understanding of the physiological responses as well as the pathophysiological changes in the brain.

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Poster

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Topic: I.04. Physiological Methods

Support: Science Fund for Creative Research Group of China (No. 61121004)

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Title: Novel skull optical clearing for *In vivo* imaging of neural circuitry in mice cerebral cortex

Authors: *D. ZHU, Y. ZHAO, C. ZHANG, T. XU;
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Abstract: *In vivo* two-photon microscopic imaging of cerebral cortex in fluorescent protein transgenic mice have shown great potential for understanding of a broad array of neurobiological phenomena, especially the dynamics of individual synapses and the functional organization of cortical maps in learning and memory and various brain diseases. Regarding the imaging performance always suffers from the turbid skull above the cortex, various cranial windows, such as removed skull, cranial window, thinned-skull cranial window, and polished and reinforced thinned skull window were developed to improve imaging performance. However, these models are not options available for infantile mice due to the vulnerability of young animals. Actually, the critical period for cortical development for young mice is always a matter of concern. The peak of anatomical and physiological plasticity of dendritic spines occurs during the third week. Up till now, the plasticity of neural infactile mice circuitry less than one month has not been studied very well. In this work, we developed an innovative skull optical clearing method, which can make the skull transparent by topically treatment with skull optical clearing agents instead of craniotomy. Through the clearing skull, we could image the dendritic protrusions of infactile mice *in vivo*. Our preliminary data showed the strong dynamics of cortex circuitry in three-week old mice. Besides, a skull optical clearing method was developed for imaging cortical neural circuitry of adult mice. In addition, the safety of skull optical clearing methods was approved by dynamical monitoring the blood flow and observation of inflammation reaction.

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Poster

560. Optical Methods: Real-Time Imaging

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Topic: I.04. Physiological Methods

Support: DFG

Einstein

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ERC

NeuroCure

Title: Taking Pixies of whiskers: Real time optical tracking of mouse behavior

Authors: L. BLANCO[1], M. A. NASHAAT[1], H. ORABY[1], M. E. LARKUM[1], *R. N. SACHDEV[2];
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Abstract: Rodents explore their environment with their whiskers. Motion of whiskers is typically monitored by EMG or by high speed videography. However EMG does not give information about individual whisker movement and high speed videography does not provide real-time information. Here we have customized and extended an off the shelf optical tracking system, the Pixy camera, and use it in a novel approach to study whisker use in mice. Head-fixed mice were trained to move their whiskers to make contact with a piezo film sensor in response to an auditory cue. Two adjacent whiskers were painted in different colors with UV fluorescent paint, and illuminated with dark / UV light. Using the Pixy camera, we tracked multiple whisker trajectory at 50 Hz (resolution of Pixy), and aligned whisker motion with cue onset and whisker contact. We simultaneously recorded mouse behavior with a color high-speed camera. The high-speed video was then played back in slow motion for Pixy based tracking of the colored whisker's trajectory. The resulting offline video data had higher temporal resolution. Our analysis of one hundred trials, 10 minutes, of both online and offline data reveals that, on average, whiskers on one side move in a highly correlated way, but the whisker in contact with the sensor shows different amplitude and dynamics than the adjacent whisker. We propose that this method can be used for simultaneous detection of multiple dimensions of behavior, including motion of the whole animal, and whisker, limb or head movement.

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Poster

560. Optical Methods: Real-Time Imaging

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Topic: A.10. Development and Evolution

Title: Development and discussion of dti phantom for nerve fiber tracking.

Authors: ***S. YOKOYAMA**, T. HIROYASU, S. HIWA;
Doshisha Univ., Kyotanabe-Shi, Japan

Abstract: [Purpose]
In this paper, the DTI-phantom which was developed for evaluating fiber tracking methods was discussed. Recently diffusion tensor fiber tracking shows many suggestions for analyzing brain functions. However, diffusion tensor fiber tracking methods have several defects and they have to be resolved. Real detailed structure of nerve fiber in brain is unknown and nerve fiber phantoms should be utilized for evaluating fiber tracking methods.
In the previous study the phantom which is consisted of Dyneema fiber whose thickness diameter is 15 [μm] was proposed. The thickness of the nerve fiber in brain is 0.2[μm] to 20[μm] in diameter. Thus, Dyneema fiber is a little thick to simulate nerve fiber.
In this research, fibers who has hollows and whose thickness is 5.4 [μm] was used for the phantom. In the experiment, the developed phantom was used to evaluate the fiber tracking methods.
[Methods]
The fiber diameter for phantom is 5.4 [μm] and the phantom fascicle was consisted of 12 million fibers. Two straight phantoms which were made of hollow fibers and Dyneema fibers were compared to measure the performance of hollow fibers.
Then kissing phantom was also developed. Kissing structure is often observed in brain and two bundled nerve fibers are touching together.
Since this two bundled fibers are kissing at one point, tracking method often tracks in wrong directions. The simple kissing phantom whose two fibers are just touched has already been proposed.
In this research, kissing phantom whose bundled fibers were touched intertwined and this structure is much more similar to real brain nerve structure.
This phantom was called meshed kissing phantom.

The simple and meshed kissing phantoms were developed and the fiber tracking algorithm was applied to the measure DIT results. The results were compared and discussed.
[Result]
The results of different types of two straight phantoms were compared. Then the performance of the phantom which was consisted of hollow fibers showed the higher ability to express the nerves.
The results of two different types of kissing phantoms showed the differences.
In the meshed kissing phantom, a lot of tracking were occurred.
This result described that the meshed kissing phantom is much more similar to real brain nerves compare to the simple kissing phantom.
[Conclusions]
In this research, DTI phantom whose fiber diameter is 5.4 [μm] which is similar to real nerve fiber and who has hollows were developed and discussed.
Then, DTI phantom which has kissing structure was also developed and measured.
From the experiments, results showed that the developed DTI phantom can be used for evaluating fiber tracking methods.

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Poster

561. Neurophysiological Approaches and Techniques

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Program#/Poster#: 561.01/NNN41

Topic: I.04. Physiological Methods

Support: Indiana University Collaborative Research Grant

Title: Persistent alterations in cognitive and cortical function following maternal deprivation

Authors: ***S. S. JANETSIAN**[1], N. M. TIMME[1], B. F. O'DONNELL[3], C. C. LAPISH[2,4,5]; [2]Psychology, [1]Indiana Univ. Purdue Univ. Indianapolis, Indianapolis, IN; [3]Indiana Univ., Bloomington, IN; [4]Stark Neurosci. Res. Inst., Indiana Univ. Sch. of Med., Indianapolis, IN; [5]Inst. for Mathematical Modeling and Computat. Sci., Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Early life trauma is a risk factor for a number of neuropsychiatric disorders, including schizophrenia (SZ) and depression. Animal models have played a critical role in understanding how adverse events early in life may evoke changes in biomarkers of altered brain function and altered behavioral phenotypes that resemble these neuropsychiatric disorders. However, since SZ

is a complex condition with a multifactorial etiology, it is difficult to model the breadth of this condition in a single animal model. Considering this, it is necessary to develop rodent models with clearly defined subsets of pathologies observed in the human condition and their developmental trajectory. The current study assessed how an early life traumatic event alters cognition and brain function to better understand what neural systems might be compromised following these events. On postnatal day (PD) 9, male rat pups were maternally deprived (MD) for 24-hours or were left undisturbed. In Exp. 1, social interaction (SI), temporal memory, and recognition memory were tested in adulthood. In Exp. 2, electrophysiological recordings were obtained from the prefrontal cortex (PFC), vertex, and temporal cortex (TC) during a paired-click paradigm to assess the effects of MD on sensory gating and shared information using information theoretic analyses. Decreased SI and impaired temporal and recognition memory were observed in MD animals. In Exp. 2, MD animals had a blunted gating response during the paired-click paradigm compared to controls, which was most pronounced in the TC. Shared information was reduced following MD when examined immediately prior to or after the click and was most pronounced between the PFC-vertex and TC-vertex. These data shed light on the underlying changes in neural processing that lead to impaired temporal/recognition memory in MD animals. Impairments in the ability of each brain region to maintain and share information in the absence of the stimulus could reflect alterations in neural processing that lead to a less robust representation of each object, thereby preventing the detection of the novel one. Importantly, these data suggest that the deficits in auditory gating are not attributable to deficits in early sensory processing but rather a systems level phenomenon that is required to map contextual information to sensory stimuli. Lastly, these data suggest that neurodevelopmental perturbation early in life was associated with long-lasting alterations in cognition and brain function in adulthood. As such, this model may provide a useful tool to further explore the neural basis of early life trauma that may result in mental psychiatric disorders, including SZ.

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Poster

561. Neurophysiological Approaches and Techniques

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Topic: I.04. Physiological Methods

Title: Functional human neurons derived from iPS cells display a range of unique and “exciting” MEA phenotypes.

Authors: *K. P. MANGAN, C. KANNEMEIER, E. ENGHOFER, J. MA, S. DELAURA, C. CARLSON;
Cell. Dynamics, Intl., Madison, WI

Abstract: As many of the underlying mechanisms in neuronal disorders and neurotoxicity affect the electrical properties of the nervous system, there is an increasing demand for more biologically-relevant cell models coupled with scalable electrophysiological techniques to understand and measure neuronal activity and network connectivity. Multi-electrode array (MEA) platforms enable electrically active excitable cell types to be monitored in real-time. Although primary rodent cortical neurons are the most widely-accepted cell source for MEA, recent advances in iPSC technology now provide access to previously unattainable human neural cell types and offer a unique source of relevant and assayable cells.
Using a panel of highly pure iPSC-derived neural cell types, we are able to detect a range of different phenotypes on the MEA platform. The catalog cell types available include GABAergic (inhibitory) cortical neurons, glutamatergic (excitatory) cortical and induced neuronal (iN) cells, midbrain dopaminergic neurons (excitatory), and astrocytes (glia). This poster focuses on MEA functionality of these cells individually or in co-culture. We find that both complex patterns of spontaneous electrical activity and the degree of synchronous bursting depend on the ratio of excitatory to inhibitory cells (the "E/I ratio") within the culture. To help illuminate this finding, we track the evolution of a synaptically-driven culture over time and analyze changes in numerous parameters that define neuronal MEA activity, such as mean firing rate, channel burst rate, or network bursting percentage. Interestingly, basal media and supplements have an extraordinary effect on MEA assay performance. Similarly, addition of astrocytes to cultures of pure neurons dramatically impacts the functional phenotype and further promotes network-level synchrony. Finally, with a range of baseline MEA phenotypes established, we tested the pharmacological effects of compounds targeting key receptors, such as GABA, AMPA, and NMDA. Overall, these human iPSC-derived neuronal cultures responded appropriately to known agonists and antagonists (e.g. bicuculline, AP5/DNQX, and kainic acid).
An imbalance in the E/I ratio in the brain is associated with numerous neurological abnormalities and deficits. Currently-used non-human cell models on the MEA are limited in their ability to broadly address different human diseases *in vitro*. The data presented here demonstrate that human iPSC-derived neuronal cell types are rising to the challenge of becoming a reliable and predictive tool for use in drug discovery, disease modeling, and neurotoxicity applications.

Disclosures: K.P. Mangan: A. Employment/Salary (full or part-time): Cellular Dynamics International. **C. Kannemeier:** A. Employment/Salary (full or part-time): Cellular Dynamics International. **E. Enghofer:** A. Employment/Salary (full or part-time): Cellular Dynamics International. **J. Ma:** A. Employment/Salary (full or part-time): Cellular Dynamics International. **S. DeLaura:** A. Employment/Salary (full or part-time): Cellular Dynamics International. **C. Carlson:** A. Employment/Salary (full or part-time): Cellular Dynamics International.

Poster

561. Neurophysiological Approaches and Techniques

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The CABMC (Control of Animal Brain using MEMS Chip) funded by Defense Acquisition Program Aministration (UD140069ID)

Title: Electrical Stimulation on Amygdala for animal's behavior control

Authors: *Y. LEE[1], Y. CHO[2], Y. LEE[2], S. KIM[2], J. LEE[2], S. JUN[2,3];
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Abstract: Stimulating the branch of the medial forebrain bundle (MFB) is frequently used to train animal's behavior, because MFB is dopaminergic pathway which makes animal feel pleasure and reward. Stimulating amygdale nucleus (AMY), the site of inducing fear, is also used for training animal by punishing animal which goes wrong direction. In this study, we used stimulations for training animal's behaviors in response to signals for direction. The effectiveness of the AMY stimulation for behavior control was compared with that of previous methods. Sprague-Dawley rats are used as experimental animals and surgery is performed to implant electrodes in their brain in order to watch animals' behaviors under freely moving condition. Each pathway is electrically stimulated with a multi-channel microelectrode array (tungsten, 254 μm). Stimulation of somatosensory cortex is used for directional cues in which direction to go. We divide three groups by electrical stimulation site standards, neural pathway of reward (Group R), fear (Group F) and both (Group B). Effectiveness can be measured by the rate of correct directional decision making, and the number of days finishing the training session until the success rate is over 75%. Our results demonstrate that using both stimulation is the best method for training animal. This study not only integrate existing methods and but also prove advanced effectiveness of integrated method. As a result, it can be expected to accelerate animal's behavior related experiments.

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Poster

561. Neurophysiological Approaches and Techniques

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Topic: I.04. Physiological Methods

Support: Darpa SBIR Phase II Contract W911NF-15-C-0069-P0002

Title: Implantable neural recording and stimulation for in-vivo electrophysiology

Authors: *J. C. MORIZIO, V. GO;
Triangle Biosystems, Inc, Durham, NC

Abstract: Over the last decade, wireless technology advancements for neural recording and stimulation continue to improve with respect to data rates, power consumption, weight and size thus inspiring new experiments for *in-vivo* electrophysiology research on freely moving rodents. In this presentation, we introduce a neural recording system using telemetric implantable capsules that can record from 5 to 64 channels of EEG, EMG, ECG, and single units or spikes signals simultaneously in real time. In addition, we present an implantable neural stimulation system which includes full duplex digital transceiver capsules that can stimulate 2 channel or 16 channel constant current bipolar pulses or 2 channel optogenetic stimulation. Key design challenges and trade-offs of these implantable wireless technologies will be explained. Sub-system components and accessories will also be described that include electrodes and neural interfaces, as well as low noise integrated CMOS electronics, RF transceiver circuitry, 90-day packaging, coating processes and inductive powering technologies. Test data and electrical specifications for each of the implantable technologies will also be presented. DAQ analysis software used for neural recording and stimulation for pattern definition and triggering will conclude the presentation.

Disclosures: **J.C. Morizio:** None. **V. Go:** None.

Poster

561. Neurophysiological Approaches and Techniques

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Title: A high-throughput system to characterize functional connectivity between cortical areas

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Abstract: Despite a thorough mapping of the anatomical connectivity between brain regions and decades of neurophysiological studies of neuronal activity within the various areas, our understanding of the nature of the neural signals sent from one area to another remains rudimentary. Orthodromic and antidromic activation of neurons via electrical stimulation ("collision testing") has been used in the peripheral nervous system and in subcortical structures to identify signals propagating along specific neural pathways. However, low yield makes this method prohibitively slow for characterizing cortico-cortical connections. We employed recent advances in electrophysiological methods to improve the efficiency of the collision technique between cortical areas. There are three key challenges: 1) maintaining neuronal isolations following stimulation, 2) increasing the number of neurons being screened, and 3) ensuring low-latency triggering of stimulation after spontaneous action potentials. Our system addresses these issues using recent improvements in on-line tetrode-based isolations, linear array electrodes, and the processing speed of data acquisition hardware systems. We have developed a software package for on-line isolations and stimulation triggering, which operates in conjunction with a Hardware Processing Platform (HPP). The HPP is a system on a chip solution enabling real-time re-programming. Employing the HPP programmable device for template matching both accelerates spike sorting and provides the low-latency triggering of stimulation required to produce collision trials. Recording with a linear tetrode array electrode allows simultaneous screening of multiple neurons, while the software package coordinates efficient collision testing of multiple user-selected units across channels. This high-throughput connectivity screening system will enable researchers working with a variety of animal models and brain regions to

identify the functional properties of specific projections between cortical areas in behaving animals.

Disclosures: **M. Parsa:** None. **M. Dehaqani:** None. **B. Mumey:** None. **C. Stengel:** None. **B. Noudoost:** None.

Poster

561. Neurophysiological Approaches and Techniques

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Title: The influence of behavior training on motor cortex activation

Authors: *J. LEE[1,2], Y. CHO[2], Y. LEE[2], S. KIM[2], S. JUN[2,3];
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Abstract: As basic research for neural prostheses or neuroscience studies, there have been many approaches to modulate the animal behaviors through artificial control. Among various ways, one of the most popular method to control animal behaviors is to stimulate the dopaminergic reward system of brain. In this study, we electrically stimulate the mesolimbic dopaminergic pathway involved in reward, pleasure and habituation. We focused to investigate the influence of habituation toward motor cortex activation throughout the several repeated training periods. The rat movement according to the stimulation, and the local field potential recorded from motor cortex during the forced moving session, is carefully observed and compared. The medial forebrain bundle (MFB) area was electrically stimulated with a tungsten stimulating electrode array (200 μm diameter). The neural activities of the motor cortex region of the rat, including hindlimb and forelimb area, are simultaneously recorded using tungsten recording electrode array (100 μm diameter) during experiments. The relationship between the stimulation of MFB and the neuronal activation of motor cortex is carefully investigated. As a result, after the repeated training session the motor cortex activation increased while the rat received the reward stimulation.

Disclosures: **J. Lee:** None. **Y. Cho:** None. **Y. Lee:** None. **S. Kim:** None. **S. Jun:** None.

Poster

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Support: NIH National Center for Advancing Translational Sciences (NCATS), grant UL1TR000427

Mathias Koch Memorial Fund

Title: Feasibility and dose tolerability of high definition transcranial direct current stimulation in healthy adults

Authors: C. A. TURSKI[1], A. KESSLER-JONES[1], B. HERMANN[1], *D. HSU[1], J. E. JONES[1], S. SEEGER[1], R. CHAPPELL[2], C. IKONOMIDOU[1];
[1]Dept. of Neurol., [2]Statistics and of Biostatistics/Medical Informatics, Univ. of Wisconsin, Madison, WI

Abstract: Transcranial direct current stimulation (tDCS) enables noninvasive electrical stimulation of the cortex via electrodes placed on the subject's skull. High definition tDCS (HD-tDCS) allows for precise generation of electrical fields over selected cortical areas using multiple electrodes. tDCS has been shown to improve motor learning, visuomotor coordination, probabilistic classification and boost memory in humans. Most studies using HD-tDCS have administered stimulation to one brain area for a small number of sessions (up to 10). The safety, feasibility and tolerability of stimulation of multiple brain regions per session using HD-tDCS over long periods of time have not been established. The purpose of this trial was to study feasibility, tolerability, and safety of HD-tDCS applied over 2-4 different brain regions at each session and administered daily for a total of 20 sessions in healthy adults. Five healthy adult subjects were recruited, 2 females and 3 males (mean age 23.4 y). Subjects underwent physical and neurological examination, electrocardiogram, electroencephalogram and IMPACT test before study initiation, during the study and at completion of the study. Four networks were stimulated using HD-tDCS, left and right temporooccipital and left and right frontal. Sessions 1-10 included stimulation of both temporooccipital networks (1 mA in week 1 and 1.5 mA in week 2 over 20 min/network). Sessions 11-15 included 20 min long stimulation of all 4 networks at 1.5 mA/network and sessions 16-20 consisted of 2 stimulation cycles on each day of all 4 networks at 1.5 mA/network. All subjects completed the trial. Adverse events reported were tingling at the site of stimulation during stimulation (5/5), transient redness at the frontal electrodes (1/5), the feeling of being stimulated for 2 hrs after one session (1/5) and one incident of headache during stimulation which resolved when stimulation was discontinued (1/5). There were no abnormalities detected on EKG, EEG, physical and neurologic examination. The scores in the

immediate post-concussion assessment and cognitive (IMPACT) test were similar before and after the 20 stimulation sessions. This pilot trial demonstrates that prolonged daily stimulation of multiple brain regions over 4 consecutive weeks using HD-tDCS is feasible and well tolerated in healthy adults.

Disclosures: **C.A. Turski:** None. **A. Kessler-Jones:** None. **B. Hermann:** None. **D. Hsu:** None. **J.E. Jones:** None. **S. Seeger:** None. **R. Chappell:** None. **C. Ikonomidou:** None.

Poster

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Topic: I.04. Physiological Methods

Title: Biomarkers of tissue damage during electrical stimulation in rat sciatic nerve.

Authors: *P. TAKMAKOV[1], D. X. HAMMER[2], C. G. WELLE[3], S. VASUDEVAN[2]; [1]CDRH-OSEL-DBCMS, [2]CDRH-OSEL-DBP, FDA, Silver Spring, MD; [3]Dept. of Neurosurg., Univ. of Colorado Denver, Aurora, CO

Abstract: There is an emerging interest in exploring neuromodulation of peripheral nervous system (PNS) for clinical applications, as it relays central command-and-control signals to almost all of the body organs. Neuromodulation of PNS is an attractive target due to relatively simple anatomy and ease of access, which enables precise targeting with a hope of fewer side effects and less invasive surgical procedures. One of the limits of electrical neuromodulation is the risk of tissue damage at high stimulation levels.
Classical studies that have documented these effects and established rules for safe electrical stimulation were conducted over half a century ago (Lilly et al., 1955) primarily in the CNS. Since then, surprisingly little progress has been made in identifying the mechanism(s) of tissue damage. Moreover, while the results obtained in CNS were translated to PNS, there is evidence that a different parameter space defines damage in PNS (McCreery et al., 1992).
In this study we evaluate neural tissue damage in rat sciatic nerve during electrical stimulation through optical, functional and histochemical measurements. Exposed sciatic nerve in anesthetized animals was stimulated over a range of charge density and charge per phase parameters (McCreery et al., 1990). The stimulation was accompanied by real-time quantitative Optical Coherence Angiography (qOCA), among other traditional behavioral and histological measures. qOCA in particular, allows observation of changes in blood vessels and to determine the potential impact of vasoconstriction on tissue damage during stimulation. Seven days post stimulation, functional damage to the nerve was characterized using walking track analysis,

followed by immunohistochemical evaluation of tissue damage. The characterization of the tissue damage in rat sciatic nerves with several biomarkers allows more comprehensive assessment of tissue response, which can be used to evaluate the safety of electrical stimulation protocols and explore the mechanisms of PNS nerve damage.

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Lilly, J.C., Hughes, J.R., Alvord, E.C., and Galkin, T.W. (1955). Brief, Noninjurious Electric Waveform for Stimulation of the Brain. Science *121*, 468-469.
McCreery, D.B., Agnew, W.F., Yuen, T.G., and Bullara, L. (1990). Charge density and charge per phase as cofactors in neural injury induced by electrical stimulation. IEEE Trans. Biomed. Eng. *37*, 996-1001.
McCreery, D.D.B., Agnew, W.F., Yuen, T.G.H., and Bullara, L.A. (1992). Damage in peripheral nerve from continuous electrical stimulation: Comparison of two stimulus waveforms. Med. Biol. Eng. Comput. *30*, 109-114.

Disclosures: P. Takmakov: None. **D.X. Hammer:** None. **C.G. Welle:** None. **S. Vasudevan:** None.

Poster

561. Neurophysiological Approaches and Techniques

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Topic: I.04. Physiological Methods

Support: NSF IGERT 1250104

Title: Magnetoelectric thin films for neural stimulation

Authors: *A. WICKENS[1], J. T. ROBINSON[2];
[1]Applied Physics, [2]Rice Univ., Houston, TX

Abstract: A method to remotely stimulate neurons would allow for the study of deep brain regions in freely moving animals and lead to potential applications in new forms of treatment for disorders such as Parkinson's disease. For clinical applications, techniques that do not require genetic manipulations are most desirable. The two primary clinically approved neuromodulation techniques used to treat neurological diseases are transcranial magnetic stimulation (TMS) and deep brain stimulation (DBS). TMS makes use of the property that magnetic fields can pass through tissue without attenuation but uses strong magnetic fields (1-3 Tesla) and is unable to specifically stimulate areas deeper in the brain. To target these areas DBS uses implanted electrodes with wires extending through the skull to deliver a local electrical signal. These wires

can cause damage to other brain areas and leave the tissue unenclosed following implantation. An ideal neuromodulation device would be enclosed in the tissue after implantation and externally activated to deliver a specific electrical signal to a deep brain region. Using the magnetoelectric coupling between a magnetostrictive and piezoelectric material we propose the use of magnetoelectric thin films as a novel neuromodulation device. This device will allow for specific less-invasive neural modulation by the application of a small external magnetic field that is transformed to a local electric field.
To create a biocompatible magnetoelectric film we bonded a piezoelectric material polyvinlydene fluoride and a magnetostrictive material Metglas. We then encapsulated the films to make them biocompatible. These films can generate voltages above three volts under resonant conditions using alternating magnetic fields with an amplitude of about 2 mT. We can also design the film geometry to resonate at different frequencies, which allows us to independently control multiple stimulation channels on a device. We have also shown these films to be biocompatible *in vitro* using cell viability assays over a period of seven days with no loss of device functionality.
In conclusion, these biocompatible magnetoelectric devices can convert magnetic fields that can penetrate the brain into electric fields that interact with surrounding cells to modulate their activity. This technology has many prospective applications including the exploration of neural networks and the modulation of neural activity in freely moving animals. Magnetoelectric materials could also be developed into a novel deep brain stimulation treatment for neural disorders like Parkinson's disease using devices that cause less neural damage compared to current electrodes.

Disclosures: A. Wickens: None. **J.T. Robinson:** None.

Poster

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Support: CIHR operating grant MOP 123247

Title: Effects of transcranial magnetic stimulation of FEF on neurophysiological activity in contralateral FEF

Authors: *S. J. LEHMANN[1], B. D. CORNEIL[2];
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Abstract: Transcranial magnetic stimulation (TMS) allows non-invasive perturbation of neural activity, induced by a rapidly changing magnetic field. Both single pulse and repetitive TMS (rTMS) modulate behavioral output in both humans and animals, including non-human primates (NHPs). Despite being an important methodology in cognitive neuroscience and offering a potential treatment for neurological disorders like neuropathic pain, depression, or stroke, a precise understanding of the effects of TMS on neural activity in an interconnected brain network, and how such effects influence behavior, is largely lacking. To overcome this gap, we are developing an animal model of TMS, focusing on the oculomotor network in NHPs. Previous work has shown that delivering single-pulse TMS to the frontal eye fields (FEF) evokes feed-forward neck muscle responses, likely through the downstream superior colliculus, that can be used to optimize TMS location over the frontal cortex, and verify an effect of TMS. Now having a reliable means of delivering TMS to the FEF, we are applying TMS either in a rapid pattern (e.g., single- or double-pulse TMS delivered at a precise time during a behavioral task), or in a repetitive pattern (e.g., 1-Hz TMS for 10-15 minutes). Our current focus is to examine the effects of TMS of one FEF on neuronal activity in the contra-lateral FEF during performance of an intermixed pro- and anti-saccade task. We present data from 14 recording sessions, using a semi-chronic multi-electrode approach. During the task, single pulse TMS-FEF was delivered at 70-75% motor-threshold (defined by TMS-intensity needed to evoke hand-muscle responses when stimulating the monkey's primary motor cortex); delivery was applied at various times of the task, ranging between 30 and 80ms before target appearance. As a control, we recorded activity from the same neurons while stimulating either primary motor cortex ("brain-control") or 3 cm above the monkey's head ("sham-control") with the same intensity. Preliminary results revealed a diversity of effects on task related single-unit spiking activity (N=58) after TMS-FEF, with TMS influencing activity following visual target presentation, and saccadic activity preceding the execution of pro- and anti-saccades. These results are further contributing to the optimization of our experimental approach, and highlight the challenges in trying to differentiate specific from non-specific effects of TMS on neuronal activity.

Disclosures: S.J. Lehmann: None. **B.D. Corneil:** None.

Poster

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Topic: I.04. Physiological Methods

Support: NIH Grant EB00856

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Title: Using electrical stimulation and recordings from the cortical surface to infer principles of cortical communication

Authors: *L. CROWTHER[1], P. BRUNNER[2], A. L. RITACCIO[2], G. SCHALK[1,2,3]; [1]Wadsworth Ctr., Albany, NY; [2]Albany Med. Col., Albany, NY; [3]State Univ. of New York, Albany, NY

Abstract: The principles that govern large-scale cortical communication are still largely unclear. To better understand these principles, it is necessary to establish anatomical connectivity between different areas in the cortex and determine how functional connectivity between these areas is affected by modulatory processes, in particular oscillatory activity. Electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) lack either the spatial or temporal resolution to accurately study these properties and can only provide correlational rather than causal evidence. In the present study, we have developed methods that combine direct electrical stimulation of the cortex with electrocorticographic (ECoG) recordings. Specifically, we apply trains of discrete electrical stimuli (300 us pulse duration, 500 ms inter-stimulus period, current amplitudes up to 15 mA) to the surface of the brain while recording cortical activity with high-density ECoG grids (inter-electrode distance 3-10 mm, center to center). To date, we have performed this procedure in 6 patients that underwent clinically indicated placement of subdural electrodes. Our methods allow us to identify the cortical locations whose population-level activity is changing as a result of the electrical stimulation, and allow us to quantify the magnitude of this effect. Ongoing work is identifying the causal role of oscillatory activity in modulating the effect of electrical stimulation. Future studies will test the hypothesis that this effect is increased during the oscillatory trough or when oscillatory power is low.

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Poster

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Support: NIH DP2-EY025446

Title: Generating complex neural patterns with multi-site electrical microstimulation

Authors: ***S. TAFAZOLI**, K. LETAI, T. J. BUSCHMAN;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Electrical microstimulation is often used as a tool for mapping sensory-motor functions and studying the neuronal basis of cognition. However, the majority of this has been through stimulation of a single site; it is largely unknown how multi-site microstimulation affects cortical microcircuits. To test this, we combine simultaneous multi-electrode recordings with multi-site stimulation in anesthetized mice. Stimulation and recording were performed with 32-channel silicon probes placed in either visual or parietal cortices. Using 16 channels for recording and 16 channels for stimulation allowed us to simultaneously record from small populations of single neurons while patterning stimulation at nearby sites. We found one can reliably elicit different patterns of neural activity across the population of record neurons, depending on which stimulation sites were activated. In addition, the dimensionality of the stimulation evoked responses was similar to that observed during baseline activity. Finally, multi-site stimulation was largely non-linear and difficult to predict from single site stimulation alone. These findings suggest multi-site microstimulation can produce a multi-dimensional neural response and provides the building block for probing complex cognitive functions and neural dynamics.

Disclosures: **S. Tafazoli:** None. **K. Letai:** None. **T.J. Buschman:** None.

Poster

561. Neurophysiological Approaches and Techniques

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Topic: I.04. Physiological Methods

Support: NIH R01 EB019005

AHA Western Affiliates Postdoctoral Fellowship

Title: Focused ultrasound at 43 MHz inhibits action potential firing in hippocampal brain slices

Authors: ***M. L. PRIETO**[1], D. V. MADISON[1], B. T. KHURI-YAKUB[2], M. C. MADUKE[1];
[1]Dept. of Mol. and Cell. Physiol., [2]Edward L. Ginzton Lab., Stanford Univ., Stanford, CA

Abstract: Ultrasound neurostimulation is a promising technology for modulating brain activity *in vivo.* To guide the development of this new technology, we seek to understand the physical

and biophysical mechanisms underlying the effects of ultrasound (US) on action potential firing. These mechanisms are poorly understood, partly because single-cell electrophysiological data on the effects of US on neural activity have been unavailable. Here we describe an experimental set-up that allows stable patch-clamp recording from brain slices in the presence of focused US stimulation at 43 MHz and intensities up to 50 W/cm^2, and we report preliminary results describing the effects of US at 50 W/cm^2 on neurons from the CA1 region of the rat hippocampus. We find that US inhibits action potential activity, reducing the number of spikes fired during a depolarizing current step while increasing the latency to the first spike and the interval between spikes. Since the effect of US on neural activity *in vivo* is typically an increase in action potential firing, this result suggests that in the context of *in vivo* brain circuits US may potentiate firing by reducing the activity of inhibitory neurons. We also find effects of US on the action potential waveform: the peak voltage is reduced and the width is decreased, while the initial rising phase is relatively unaffected. In addition, a slight reduction of the steady voltage level during the current step is apparent during the US stimulus when several trials are averaged. Together, these results suggest the hypothesis that US increases the activity of Ca^{2+}-activated K^+ channels, which play a prominent role in determining action potential width. US could activate these channels directly, or indirectly through increased activity of Ca^{2+} channels. We will report on the results of experiments to test this hypothesis along with additional experiments to determine the physical modality through which US affects neural activity.

Disclosures: **M.L. Prieto:** None. **D.V. Madison:** None. **B.T. Khuri-Yakub:** None. **M.C. Maduke:** None.

Poster

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Support: NIH Grant R21 NS082870

Title: Effect of iTBS on cortical and corticospinal excitability in healthy subjects: a TMS-EEG-EMG study

Authors: *T. GEDANKIEN, P. J. FRIED, A. PASCUAL-LEONE, M. SHAFI;
Neurol., Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: Noninvasive brain stimulation techniques, such as Transcranial Magnetic Stimulation (TMS) and intermittent Theta Burst Stimulation (iTBS), enable the characterization of cortical

and corticospinal reactivity in humans. TMS coupled with electromyography (EMG) allows for the recording of motor evoked potentials (MEPs), while TMS and encephalography (EEG) permit recording of TMS-evoked EEG potentials (TEPs). While it has been shown that TBS over the primary motor cortex (M1) can induce changes in cortical and corticospinal reactivity, the relationship between such TBS-induced changes remains poorly understood. In this study, we examine the correlation between MEPs and early TEPs from single-pulse TMS before and after iTBS to the left M1 in 8 healthy subjects. TMS was targeted to the hand region of M1 using the MRI-guided Nexstim eXimia navigated brain stimulation system and a Nexstim eXimia figure-of-eight biphasic coil. MEPs were recorded from the first dorsal interosseous (FDI) of the right index finger via Ag-AgCl surface electrode-pairs. TMS single pulses were delivered at 120% of the resting motor threshold (RMT; 5/10 $\geq$ 50 μV). EEG was recorded with a 60-channel TMS-compatible EEG device, and impedances were kept below 5kΩ. A passive-cooling figure-of-eight coil attached to a MagPro X100 stimulator was used to deliver iTBS (total of 600 pulses) at 80% of active motor thresholds (AMT; 5/10 $\geq$ 200 μV during isometric contraction). Prior to and following iTBS, participants received 3 blocks of 30 TMS single pulses at 120% RMT. Peak-to-peak amplitudes of each recorded MEP and TEP were averaged for each subject. Of the 8 subjects, 4 demonstrated an MEP increase >50% after iTBS, and are labeled “MEP responders”. The other 4 showed MEP change of <10%, and are termed “MEP non-responders.” In the 4 MEP responders, there was a significant increase in the magnitude of the TEP P30 amplitude (mean pre = 4.1 μV; mean post = 6.1 μV; $p < 0.001$). In contrast, in the 4 MEP non-responders, there was no significant change in TEP P30 amplitude (mean pre = 3.53 μV; mean post = 1.8 μV; $p > 0.2$). Across all 8 subjects, the percentage change in TEP P30 amplitude was significantly correlated with the percentage change in MEP amplitudes ($r=0.82$; $p < 0.05$), suggesting that iTBS-induced changes in cortical and corticospinal plasticity are linked. Our results demonstrate that when iTBS induces changes in corticospinal reactivity, cortical reactivity is similarly modulated. The changes in EEG measures of cortical reactivity are correlated with conventional MEP changes. Going forward, concurrent TMS-EEG may be used to directly evaluate cortical plasticity mechanisms in non-motor brain regions.

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Poster

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Support: NIH 5R01NS089679-02

NIH 5U01NS090454-02

Title: A multi-channel electrode for chronic recording and safe current-steered stimulation

Authors: *B. PEARRE[1], S. MOORMAN[2], J. SHEN[3], T. GARDNER[3];
[1]Biol., Boston Univ., Cambridge, MA; [2]Biol., [3]Boston Univ., Boston, MA

Abstract: Electrical control of the brain facilitates a variety of therapeutic and scientific goals, from treating sensory, motor, and cognitive defects to exploring the effects of disrupting or subtly modifying the brain's behaviour in real time. These procedures are limited by the brain's reaction to foreign matter: over a period of months, glia encapsulate the electrodes, isolating them from neurons, allowing monitoring and control of the brain only over large spatial scales---often on the order of millimeters. Small electrodes (< 10 um) minimise encapsulation, and thus can both record single neurons for many months and precisely stimulate small groups of neurons. However, the high impedance of small electrodes can require stimulation voltages that exceed the water hydrolysis point.

We have developed an electrode design in which groups of electrodes support each other during insertion and then splay randomly in the brain, allowing long-term small-spatial-scale recording and stimulation [Guitchounts 2013].

We describe the splaying properties of these electrode arrays in the brain. We present preliminary results showing that these electrodes remain capable of recording individual spikes for a year after implantation, even when also used to stimulate.

We present preliminary evidence that the spatial scale of the splaying is sufficient to allow the steering of current between the electrodes, and that this allows a degree of high-dimensional control over the brain's response to stimulation. We show that appropriate control of the electrode array can produce neural responses while keeping stimulation voltages below safety limits. Furthermore, we demonstrate controllable differentiation between responses, even in an upstream brain area.

Thus these multichannel, spatially distributed, micron-scale arrays allow long-term single-unit recordings, enabling new experiments investigating how the brain changes on long timescales. In addition, current-steered control of stimulation inputs allows fine-grained control over small groups of neurons, permitting a wide variety of optimisations, such as controlling the brain to some set of desired responses, or minimising the voltage or energy required in order to achieve the desired result.

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Poster

561. Neurophysiological Approaches and Techniques

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Topic: I.04. Physiological Methods

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Title: Types of neurons recruited and activity patterns elicited by single pulse transcranial magnetic stimulation in motor cortex of alert rhesus macaques

Authors: *M. V. SMITH[1], J. KIM[1,6], E. M. GRIGSBY[7], N. GOSWAMI[1], D. WONG[1], J. M. BERNABEI[1,6], A. V. PETERCHEV[1,2,3], W. M. GRILL[1,3,4], M. A. SOMMER[1,4,5]; [1]Biomed. Engin., [2]Psychiatry and Behavioral Sci., [3]Electrical and Computer Engin., [4]Neurobio., [5]Ctr. for Cognitive Neurosci., Duke Univ., Durham, NC; [6]Bioengineering, Univ. of Pennsylvania, Philadelphia, PA; [7]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Transcranial magnetic stimulation (TMS) is a safe, non-invasive method of brain stimulation that induces a transient electric field in cerebral cortex. Its precise effects on single neuron activity are unknown, however, which limits the rational design of TMS protocols. Here we recorded from the primary motor cortex (M1) of two alert rhesus macaques while simultaneously delivering single pulses of TMS from a MagStim Rapid2 stimulator. TMS was applied at varying intensities (10%-90% of maximum machine intensity in 10% increments) using custom coils (Mueller et al. 2014) in two configurations: “stim” TMS to induce a focal electric field at the recording site, and “sham” TMS to replicate auditory and cutaneous effects while inducing minimal electric field at the recording site. Our aims were to quantify the dose-response curves to TMS across several dimensions of neuronal response, the categories of neural response patterns to TMS, and the differential responses of inhibitory vs. excitatory neurons. We previously showed that the population response of M1 neurons to TMS is a transient burst of excitation followed by a pause in activity and that the incidence of this “canonical” response increases with TMS intensity (Grigsby et al. SfN 2015). Building on that result, we analyzed the stim and sham dose-response curves across multiple dimensions of the response, including the burst phase, the pause phase, the initiation time, and degree of trial-to-trial synchronization. In each dimension, stim vs. sham differences appeared near motor threshold (~60% machine intensity). We quantified the categories of response patterns using k-means clustering and found several distinct, yet consistent, clusters of activity profiles. In addition to the canonical response, many neurons showed prolonged excitation or inhibition following TMS. Finally, using Gaussian mixture models and related methods on the peak widths and depolarization rates of action potential waveforms, we sorted our dataset into putative excitatory neurons, putative inhibitory neurons, and putative axons. All were activated by single pulse TMS, but response

profiles differed between neural elements in distinct ways, e.g. briefer pauses for inhibitory neurons than excitatory neurons. In summary, the uniform population response of M1 neurons arises from myriad individual neural response patterns that individually obey dose-response relationships, fall into distinct categories, and are associated to some extent with putative cell type. The results inform a more mechanistic understanding of the effects of TMS in the primate brain.

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Poster

561. Neurophysiological Approaches and Techniques

Location: Halls B-H

Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 561.17/OOO5

Topic: I.04. Physiological Methods

Support: United States Food & Drug Administration Center for Tobacco Products, contract # HHSF223201510026C

Title: Selective inhibition of nicotinic acetylcholine receptor subtypes by chemical additives used in tobacco and nicotine delivery products

Authors: Y. A. KURYSHEV[1], C. WU[1], *N. B. FEDOROV[1], C. KNIGHT[1], G. E. KIRSCH[1], C. S. LEGGETT[2], A. L. MOTTER[2], M. S. ORR[2], L. C. ARMSTRONG[1];
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Abstract: The effects of nicotine on the mammalian central and peripheral nervous system are mediated via activation of nicotinic acetylcholine receptors (nAChRs). The positive reinforcing effects of nicotine appear to be mediated primarily by the α4β2 subtype, and secondarily by α6-containing subtypes, due to their preferential expression in the regions of the brain responsible

for reward. Flavorings and odorants added to nicotine delivery products might also modulate nicotine's effects on nAChRs. Menthol, a coolant commonly added to tobacco products, has been shown to augment the biological effects of nicotine, paradoxically by antagonizing nicotine's activity at certain nAChRs. To screen more efficiently for modulatory effects of additives, we have developed high throughput automated electrophysiology assays, on the IonWorks Barracuda™, for the α4β2, α3β4, α3β4α5, α6/3β2β3 and α7 nAChRs. We employed these assays to analyze the agonist and antagonist effects of 70 commonly used flavorants and odorants added to cigarettes, smokeless tobacco products and e-cigarette liquids. We detected significant antagonist activity (IC_{50} values < 100 μM on at least one subtype) with 20 of those compounds. Notably, farnesene and farnesol, which impart a green apple flavor, were strongly inhibitory, with farnesol exhibiting broad effects and farnesene selectively inhibiting α6/3β2β3. Consistent with previous studies, we found menthol to inhibit nAChR activities with IC_{50} values from 25 μM (α4β2) to 110 μM (α7). Other potent and broadly inhibitory tobacco additives were nonivamide (IC_{50} values from 1.1 μM at α7 to 9.5 μM at α6/3β2β3) and γ-dodecalactone (IC_{50} values from 7.8 μM at α4β2 to 30 μM at α7). The citrus odorants citronellal, citronellyl acetate and citronellyl formate, inhibited α4β2 with moderate potency (IC_{50} values of 28 to 35 μM) and the other nAChRs more weakly. The differential potencies of these compounds on different nAChR subtypes have the potential to influence the rewarding properties of nicotine.

Disclosures: **Y.A. Kuryshev:** None. **C. Wu:** None. **N.B. Fedorov:** None. **C. Knight:** None. **G.E. Kirsch:** None. **C.S. Leggett:** None. **A.L. Motter:** None. **M.S. Orr:** None. **L.C. Armstrong:** None.

Poster

561. Neurophysiological Approaches and Techniques

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 561.18/OOO6

Topic: I.04. Physiological Methods

Support: NIH-R01 41771-00 05

Title: Dendritic effects of theta-burst induced plasticity during direct current stimulation

Authors: ***D. LING**[1], G. KRONBERG[2], A. RAHMAN[3], M. BIKSON[3], L. PARRA[3]; [1]Biomed. Engin., The City Col. of New York, Oakland Gardens, NY; [3]Biomed. Engin., [2]The City Col. of New York, New York, NY

Abstract: Transcranial direct current stimulation (tDCS) is a noninvasive technique that uses scalp electrodes to apply weak constant current to treat a multitude of neurological disorders.

Despite widespread adoption, little is known about the physiological mechanisms by which long term effects are achieved. Electric fields are known to cause local depolarization and hyperpolarization of neuronal compartments. tDCS is therefore thought to act via polarization of neuronal somas, leading to changes in excitability. However, our group has previously shown that DCS effects on long-term potentiation (LTP) and depression (LTD) can be dependent on dendritic location, implying a role for dendritic membrane polarization. In this previous work we used constant trains of synaptic activity to induce plasticity. However, other common plasticity induction protocols are thought to better mimic in vivo synaptic activity and operate via different cellular machinery. Here we show that DCS also modulates LTP induced by theta-burst stimulation (TBS) in CA1 of rat hippocampal slices. By examining these effects in different dendritic compartments we extend our previous work on the role of dendritic membrane polarization in determining these effects. This study highlights the importance of considering both the pattern of underlying synaptic activity and the role of dendritic effects in determining long-term tDCS outcomes.

Disclosures: D. Ling: None. **G. Kronberg:** None. **A. Rahman:** None. **M. Bikson:** None. **L. Parra:** None.

Poster

561. Neurophysiological Approaches and Techniques

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Program#/Poster#: 561.19/OOO7

Topic: I.04. Physiological Methods

Support: DARPA SUBNETS program

An award from Simons Collaboration on the Global Brain

Title: Large-scale circuit mapping in macaque mesolimbic and basal ganglia systems with multi-site patterned intracortical microstimulation

Authors: *S. QIAO, K. A. BROWN, B. FERRENTINO, B. PESARAN;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Existing approaches to treat neuropsychiatric disorders, such as surgery and medications, can often help alleviate the worst effects of symptoms, but they rarely offer precise, effective and personalized therapeutic solutions and suffer side effects. Psychiatric disorders, such as treatment-resistant depression and anxiety, have diverse cognitive and behavioral components, reflecting dysfunction distributed across multiple brain regions in interconnected

networks. The development of novel neurotechnologies for treating these disorders depends critically on understanding and manipulating the dynamics of neural circuits across large-scale brain networks. To meet this need, we developed a large-scale custom-designed semi-chronic stimulation and recording microdrive device to precisely target and modulate brain regions in cortical and subcortical reward processing networks of the non-human primate (NHP) performing a reward-guided reinforcement learning and decision-making task. The device provides a flexible platform for exploring and mapping detailed functional connectivity between these brain regions via open-loop multi-site patterned intracortical microstimulation (ICMS). We implemented a temporal and spatial multi-site patterned ICMS framework combining single-pulse and tetanic (100 or 200 Hz, 50 ms-10 min) stimulation to specifically investigate the neuronal dynamics and functional connectivity of the brain regions related to the orbitofrontal cortex (OFC). We illustrate this ICMS framework by mapping and manipulating the functional connectivity across some putative pathways between the lateral OFC (lOFC) and caudate nucleus (CN), anterior cingulate cortex and CN, and lOFC and external globus pallidus (GPe). We found that a 250 ms, 200 Hz bipolar tetanic stimulation (20 μA, 100 μs biphasic charge-balanced square waveform) in the corpus callosum near the CN was able to suppress the neural responses in the CN evoked by the bipolar single-pulse stimulation (30 μA, 100 μs) in the lOFC. We also observed enhanced neural responses in the GPe due to the bipolar single-pulse stimulation (40 μA, 100 μs) applied at a node in the lOFC after a 500 ms, 100 Hz bipolar tetanic stimulation (30 μA, 100 μs) delivered at another node in the lOFC. This work offers new insights into how the effects of electrical stimulation at particular nodes, frequencies, and durations will cascade throughout the mesolimbic and basal ganglia reward pathways. The results have implications for the rational design of therapeutic patterns of closed-loop stimulation based on a circuit-level understanding of the neuropsychiatric conditions.

Disclosures: **S. Qiao:** None. **K.A. Brown:** None. **B. Ferrentino:** None. **B. Pesaran:** None.

Poster

561. Neurophysiological Approaches and Techniques

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 561.20/OOO8

Topic: I.04. Physiological Methods

Support: Craig H. Neilsen Foundation

Title: Comparison of two types of neuromuscular electrical stimulation for the reduction of contraction-fatigue of the quadriceps muscle.

Authors: *F. CLAVERIA-GONZALEZ[1,2], R. KASSAM[1], T. BARSS[1], D. COLLINS[1,3]; [1]Physical Educ. and Recreation, [2]Rehabil. Med., [3]Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Introduction: Neuromuscular electrical stimulation (NMES) can produce contractions to increase functionality and reduce secondary complications for people with spinal cord injury. Unfortunately, the benefits of NMES are limited by rapid contraction-fatigue. A promising new approach developed to reduce contraction fatigue is sequential NMES (sNMES). sNMES involves rotating stimulation pulses between multiple electrodes over a muscle belly. Accordingly, different subpopulations of motor units are recruited by each stimulating electrode which reduces motor unit discharge frequencies, metabolic demand and contraction fatigue. The most common way to deliver sNMES involves five electrodes; four cathodes over the proximal quadriceps femoris muscles (Q) and a common “fixed” anode located proximal to the knee (FIX sNMES). Presently we test a modified form of sNMES which involves four electrodes over the Q muscle bellies. Instead of having a fixed anode, the anode and cathode are rotated among the four electrodes in a "chasing" pattern (CHASE sNMES). We think that this rotation of anode and cathode will recruit Q motor units more selectively and with less overlap than FIX sNMES. Therefore, we hypothesized that CHASE sNMES will reduce contraction-fatigue of the Q, compared to FIX sNMES. Methods: Four human participants (2 males and 2 females; 27.5±7.7years) were recruited. The two types of sNMES were tested in different sessions on separate days. Each session incorporated a fatigue protocol consisting of 170 contractions (0.3 s "on", 0.7 s "off"). Contraction-fatigue was quantified by the fatigue index (FI: peak torque during the last five contractions divided by the peak torque during the first five contractions multiplied by 100). A dependent T-test was performed to test for statistical differences between FIs for each NMES condition (FIX versus CHASE sNMES). Results: Initial results showed no significant difference in FI between FIX sNMES and CHASE sNMES (71±11.5, and 69±11.1 respectively). Accordingly, by the end of the fatigue protocol FIX sNMES and CHASE sNMES resulted in no difference in contraction-fatigue. Conclusion: Therefore, CHASE sNMES may be preferred during rehabilitation since four electrodes are easier to apply and it reduces costs than 5 electrodes (FIX sNMES). In addition, another important factor that should be considered for application to clinical settings is discomfort during sNMES. Therefore, our next step will to compare this outcome between FIX sNMES and CHASE sNMES.

Disclosures: F. Claveria-Gonzalez: None. **R. Kassam:** None. **T. Barss:** None. **D. Collins:** None.

Poster

561. Neurophysiological Approaches and Techniques

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Topic: I.04. Physiological Methods

Support: Queen Elizabeth II Graduate Scholarship

Title: Activity-dependent axonal hyperpolarization contributes to neuromuscular electrically-induced contraction fatigability

Authors: *M. J. LUU[1,2], K. E. JONES[1,2], D. F. COLLINS[1,2];
[1]Fac. of Physical Educ. and Recreation, [2]Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada

Abstract: There are numerous benefits in using neuromuscular electrical stimulation (NMES) as a rehabilitation tool to improve the quality of life for those living with a neurological disease or motor-impairment. Unfortunately, these benefits are limited by rapid muscle contraction fatigability. Contraction fatigability during NMES is explained, in part, by decreases in excitability of motor axons (axonal hyperpolarization) under the stimulating electrodes, resulting in fewer motor units responding to the NMES. When this occurs, the threshold current required to produce a target compound muscle action potential (CMAP) increases. The present study was undertaken to determine the time course and extent to which axonal hyperpolarization contributes to contraction fatigability when stimulating at different frequencies. Seven healthy participants took part in this study. In separate sessions, NMES was delivered at 20, 40, or 60 Hz to the common peroneal nerve to generate 480 contractions of the tibialis anterior (TA) muscle over 8 min. Contraction fatigability was measured by the decline in torque over time. Axonal hyperpolarization was measured as changes in the threshold current required to produce a CMAP of ~30% of maximum. Threshold current was measured every second, before and during the 8 min of NMES. NMES was delivered at an intensity larger than the current tracking pulse in order to ensure that only fatigued populations of axons were being tested. Single supramaximal pulses were delivered over the TA to measure the peak twitch torque during NMES. A multiple regression analysis was done to determine the relative contributions of nerve and muscle to the declining torque output. Results show that there was an increase in threshold current during NMES due to axonal hyperpolarization, and the magnitude and rate of change was frequency-dependent. The largest current increase occurred with 40 Hz NMES, and the smallest with 20 Hz NMES. Threshold current increased faster with 60 Hz NMES and slower with 20 Hz NMES. Contraction fatigability was strongly predicted by changes in threshold current at all frequencies, and less so by changes in the muscle's maximal torque output. Axonal hyperpolarization plays a role in NMES-induced contraction fatigability and the magnitude and time course of this change

is frequency dependent. The results of these experiments provide insight into the biophysical changes that occur in axons during NMES and may be useful to improve the efficacy of NMES by minimizing contraction fatigability. This is particularly important for those individuals that use it as a therapeutic tool, thereby helping to improve their quality of life.

Disclosures: M.J. Luu: None. **K.E. Jones:** None. **D.F. Collins:** None.

Poster

561. Neurophysiological Approaches and Techniques

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Topic: I.04. Physiological Methods

Support: CIHR Graduate Scholarship

CNPq Brazil

Undergraduate Research Initiative (University of Alberta)

Craig H. Nielsen Foundation

CIHR Fellowship

Title: Contraction fatigue associated with three types of neuromuscular electrical stimulation delivered to produce a range of contraction amplitudes

Authors: *E. N. AINSLEY[1,2], S. K. HARING[1], D. J. MILLER[1], M. J. WIEST[1], A. J. BERGQUIST[3], D. F. COLLINS[1,2];
[1]Fac. of Physical Educ. and Recreation, [2]The Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada; [3]Toronto Rehabil. Inst., Toronto, ON, Canada

Abstract: Neuromuscular electrical stimulation (NMES) can be applied over a muscle belly (mNMES) or nerve trunk (nNMES) to produce contractions for rehabilitation. However, rapid contraction fatigue, which manifests as a decline in torque over time, often limits its effectiveness. This fatigue is due, in part, to the high discharge rates of motor units during stimulation. To minimize fatigue, we developed interleaved NMES (iNMES), which involves alternating stimulating pulses between the mNMES and nNMES sites, thereby reducing motor unit discharge rates. The purpose of the present experiments was to compare the fatigue associated with nNMES, mNMES and iNMES of the tibialis anterior (TA) muscle at various torque amplitudes in order to generate a simulation protocol to minimize fatigue when using

NMES. This experiment is ongoing, with iNMES data being currently collected (not presented in this abstract). Each of 9 healthy subjects participated in 12 sessions with each session conducted on separate days. Within each session, participants initially performed 2 maximum isometric voluntary contractions (MVC) of ankle dorsiflexion. Trains of stimulation (duration = 0.3s; pulse width = 500μs; frequency = 40 Hz) were delivered and stimulation amplitude was adjusted to generate either 5%, 15% or 25% of a MVC or the maximal tolerated stimulation amplitude (Max). A fatigue protocol consisting of 170 trains for one of the 4 contraction amplitudes was then conducted. Contraction fatigue was quantified by the fatigue index (FI) where FI was calculated as the average torque of the last 10 trains of the fatigue protocol divided by that during the first 10 trains. In this way, a smaller fatigue index indicates more fatigue. The FI's for mNMES at 5%, 15%, 25% and Max were 0.85±0.26%, 0.77±0.14%, 0.69±0.11% and 0.65±0.11%, respectively. The FI's for nNMES at 5%, 15%, 25% and Max were 1.07±0.48%, 0.82±0.13%, 0.78±0.22% and 0.61±0.17%, respectively. Qualitatively, larger contractions resulted in greater contraction fatigue which is consistent with the theory that motor unit recruitment during NMES follows Henneman's size principle. We anticipate that iNMES will generate less fatigue than mNMES and nNMES during low contraction amplitudes and will have a similar level of fatigue in comparison to mNMES and nNMES during larger contraction amplitudes. The results of this study have important implications for in reducing fatigue during NMES for rehabilitation.

Disclosures: **E.N. Ainsley:** None. **S.K. Haring:** None. **D.J. Miller:** None. **M.J. Wiest:** None. **A.J. Bergquist:** None. **D.F. Collins:** None.

Poster

561. Neurophysiological Approaches and Techniques

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Topic: I.04. Physiological Methods

Support: NIH Grant R01-DC011580

NIH Grant R01-GM109086

Title: Behavioral discrimination of channel specific microstimulation for central auditory neuroprostheses

Authors: ***R. VERNER**, E. BARTLETT;
Biomed. Engin., Purdue Univ., West Lafayette, IN

Abstract: Neuroprostheses offer the potential to restore a modicum of sensation back to individuals in need. One major way in which sensation is restored consists of placing electrodes to enable spatial segregation along the one or two-dimensional axes representing the sensory epithelium and fundamental stimulus dimension, namely, frequency along the cochlea for hearing or skin region for somatosensation. Part of the reason for the ongoing success of the cochlear implant is its ability to adequately stimulate specific regions along the tonotopic axis of the cochlea, allowing for adjacent channels to stimulate groups of neurons in different frequency bands, though with partial overlap. Moving more centrally from the cochlea, the tonotopic axis remains, but the way in which electrical stimulation permits behavioral discrimination is poorly understood. For example, Auditory Brainstem Implants have shown reduced efficacy for behavioral perception as compared to cochlear implants, are far more difficult to implant, and occasionally stimulate undesirable nearby structures (Brackmann et al. 1993). Primary auditory cortex (A1) may be a more desirable stimulation target due to the ease of access and low risk of stimulating non-auditory regions. A1 stimulation may also serve as a model for other sensory cortical neuroprostheses and central neuroprostheses, where there are multiple cell types and intra-areal microcircuits, such as the cortical columnar microcircuits. To understand the potential for cortical prostheses and potential bases for perceptual variability, we examined interchannel discrimination in a rat behavioral model. Our data suggest rats can discriminate between stimuli presented on adjacent electrode sites separated by 125 microns (d'=2.15, s.d. 0.24) in layer 4. When repeated in layers 2-3, rats failed to discriminate between sites even up to 375 microns apart, which was the limit of our array (d'=0.32, s.d. 0.75). This result suggests that layer 4 cortical microstimulation has the potential to generate fine resolution across a stimulus dimension. Given this, careful consideration of local circuitry, stimulation parameters, and regions targeted by microstimulation can overcome many of the performance obstacles typically seen with central neuroprostheses.

Disclosures: R. Verner: None. **E. Bartlett:** None.

Poster

561. Neurophysiological Approaches and Techniques

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Time: Tuesday, November 15, 2016, 8:00 AM - 12:00 PM

Program#/Poster#: 561.24/OOO12

Topic: I.04. Physiological Methods

Title: Effects of activity-dependent micro-stimulation on cortical activity: implications for neuro-rehabilitation studies

Authors: *A. AVERNA[1], D. GUGGENMOS[2], C. DUNHAM[2], S. BARBAY[2], G. VAN ACKER[3], V. PASQUALE[1], M. CHIAPPALONE[1], R. NUDO[3];
[1]Neurosci. and Brain Technologies (NBT), Inst. Italiano Di Tecnologia (IIT), Genova, Italy; [2]Cortical Plasticity Lab. Landon Ctr. on Aging, [3]Dept. of Physiol., Landon Ctr. on Aging, Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Enhancing functional motor recovery after localized brain injury is a widely recognized priority in healthcare as disorders of the nervous system causing motor impairment, such as stroke, are among the most common causes of adult-onset disability. Restoring the physiological function of a dysfunctional brain to improve the quality of life is a primary challenge in scientific and clinical research and will be driven through innovative therapeutic approaches. Recently, techniques using brain stimulation methodologies have been employed to promote post-injury neuroplasticity for the restitution of motor function. One type of stimulation, activity-dependent stimulation (ADS), has been shown to modify existing functional connectivity within either healthy or injured cerebral cortex and has been used to increase motor recovery following cortical injury. The aim of the present work is to investigate the ability of intracortical ADS to potentiate functional connectivity between *distant* cortical locations in the healthy brain within a single four-hour recording session in anesthetized rats. Spontaneous and evoked activity within layer V of rat pre-motor cortex (rostral forelimb area, RFA) was extracellularly recorded using a 16-contact microelectrode and analyzed following treatment of ADS driven by RFA activity or Gaussian-distributed (i.e., Random) stimulation approximating the ADS frequency. Two areas within S1 (forelimb or barrel field, FL or BF) were chosen alternately for stimulation to assess the efficacy of the stimulation on different cortical locations. We quantified first-order statistics of sorted neurons (e.g., mean firing rate and inter-spike interval distribution) and estimated functional connectivity within RFA by using cross-correlation related measures as well as the coupling of each recorded neuron to the population activity (Okun *et al.*, 2015). All stimulation protocols induce changes in the subsequently recorded activity of RFA. Our results suggest that stimulation of either BF or FL area induces differential changes in RFA activity, which may be the result of different degrees of anatomical connectivity between RFA and the two sensory regions. ADS is be more effective than Random stimulation, further strengthening the idea that ADS can be used to modulate cortical connectivity and is potentially a powerful tool to enhance neuroplasticity after injury. These investigations are critical for understanding the neurophysiological effects of ADS, as well as providing a foundation for assessing the optimal stimulation parameters for treatment following brain injury.

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Poster

561. Neurophysiological Approaches and Techniques

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Topic: I.04. Physiological Methods

Support: 2014 NASA Michigan Space Grant Consortium Undergraduate Fellowship

2016 NASA Michigan Space Grant Consortium Graduate Fellowship

2016 National Defense Science and Engineering Graduate Fellowship

Title: Using experimentally informed neuron models to find optimal neural stimuli in the medicinal leech

Authors: *A. C. FERGUSON[1], D. A. MILLER[1], M. ELLINGER[1], J. JELLIES[2], M. E. KOELLING[3], C. L. LINN[2];
[1]Dept. of Electrical and Computer Engin., [2]Dept. of Biol. Sci., [3]Dept. of Mathematics, Western Michigan Univ., Kalamazoo, MI

Abstract: Optimal control techniques can be used to find reduced energy input current stimuli that produce a desired neuron membrane voltage response. The technique in [1, 2] produces a range of stimuli which balance tracking error and input current stimulus energy. Optimal stimuli produce similar membrane voltage responses as those produced by higher energy stimuli both in simulations and in experimental work [3]. In [3], lower energy optimal stimuli were found *a priori* using models based on qualitative behavior of neurons; in the work presented here, model parameters are adjusted at the electrophysiology rig based on membrane voltage responses of a leech neuron to input stimuli. Optimal control is then applied to this experimentally informed model to find and apply optimal stimuli during the experimental session. The effectiveness of input current stimuli found using an experimentally informed neuron model is examined and compared to non-optimal stimuli as well as stimuli computed *a priori*.
References:
[1] M. E. Koelling, D. A. Miller, M. Ellinger, F. L. Severance, and J. Stahl, “Current Stimuli That Provide Membrane Voltage Tracking in a Six Dimensional Neuron Model,” *ASME Journal of Dynamic Systems, Measurement, and Control*, vol. 135, July 2013.
[2] M. Ellinger, M. E. Koelling, D. A. Miller, F. L. Severance, and J. Stahl, "Exploring optimal current stimuli that provide membrane voltage tracking in a neuron model," *Biological Cybernetics*, vol. 104, pp. 185-195, March 2011.
[3] D. A. Miller, M. Ellinger, J. Jellies, A. C. Ferguson, C. L. Linn, M. E. Koelling, “Generating neuron membrane action potentials using optimal input current stimuli in the medicinal leech,”

Program No. 268.25, Neuroscience Meeting Planner, Washington, DC: Society for Neuroscience, 2015. Online.

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Poster

561. Neurophysiological Approaches and Techniques

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Program#/Poster#: 561.26/OOO14

Topic: I.04. Physiological Methods

Support: CABMC(Control of Animal Brain using MEMS Chip) Grant funded by Defense Acquisition Program Administration UD140069ID

Title: Sinusoidal electrical stimulation to modulate neural activity in freely behaving animals

Authors: ***Y. A. CHO**, Y. LEE, Y. LEE, J. LEE, S. JUN;
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Abstract: Electrical pulse is commonly used in neuroprostheses in order to modulate neural activities. However, there have been well-known issues of rectangular waveform stimulation in terms of both electrode corrosion and tissue damage. We attempted to improve those drawbacks using sinusoidal stimulation instead of pulse stimulation. Recent in vivo studies have revealed that the performance of sine wave stimulation was significantly more efficient than biphasic pulse stimulation limitedly in retina experiments. Our previous study also presented that electrical sinusoidal stimulation has advantages not only in the effective stimulation but also in the ease of artifact removal using a filter with corresponding frequency. In the present study, electrical sine wave stimulation was applied to a freely moving animal to observe neural activities in several brain areas. Manually manufactured stimulation electrodes (180m diameter) were implanted in the ventral tegmental area (VTA), and recording electrodes (100m diameter) were inserted in both nucleus accumbens (NAc) and primary motor cortex (M1). Local field potentials (LFPs) was investigated to show the difference of power discharged before and after VTA stimulation. In addition, long-term stimulation using sinusoidal waveforms and pulse train will be experimented for immunohistological analysis. This study is anticipated to show that the sinusoidal waveform stimulation is electrode- and tissue-protective method.

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Poster

561. Neurophysiological Approaches and Techniques

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Program#/Poster#: 561.27/OOO15

Topic: C.09. Brain Injury and Trauma

Support: Psychiatry Practice Plan MUHC

Title: Targeting the therapeutic properties of cannabidiol: focus on depression and pain.

Authors: G. GOBBI[1], R. MCLAUGHLIN[1,3], *D. DE GREGORIO[2], R. OCHOA-SANCHEZ[1], L. LUONGO[4], S. MAIONE[4];
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Abstract: Background: Recently, there has been an increased interest in exploiting the therapeutic properties of cannabis and its derivatives, even if their precise therapeutic indications have not yet been completely elucidated in preclinical and clinical studies. Cannabidiol (CBD), the primary non-psychotropic constituent of cannabis, is thought to have antidepressant and analgesic effects (Metchoulam et al., 2007, Chem Biodivers. Aug;4(8):1678-92), *via* a multi-target mechanism of action, including the serotonergic 5-HT1a subtype receptor (Rock et al., 2012, Br J Pharmacol. Apr;165(8):2620-34). The aim of this study was to investigate the effect of CBD on 5-HT neurons of Dorsal Raphe Nucleus (DRN), attempting to further characterize its presynaptic serotonergic mechanism involved in both allodynia/hyperalgesia and in depression-like behavior. **Methods:** Using *in vivo* electrophysiology, we studied the effect of acute (0.05-1.0 mg/kg, i.v.) and chronic (5.0 mg/kg/day) treatment with CBD on 5-HT DRN electrical activity in adult male Sprague-Dawley rats (PND 70). The 5-HT_{1A} receptor antagonist WAY100635 (0.3 mg/kg, i.v.) and the CB_1 receptor antagonist AM251 (1.0 mg/kg, i.v.) were also injected prior to CBD. Forced Swim Test (FST) and spared nerve injury (SNI) were also done to test the antidepressant and analgesic properties of CBD, respectively. Dynamic Plantar Aestesiometer and Plantar Test were employed to test the anti-allodynic and anti-hyperlagesic effect, respectively after 3, 7 and 14 days post SNI. **Results**: Cumulative injection doses of 0.1-1.0 mg/kg led to a decrease in 5-HT DRN firing activity ($P<0.001$, n=9). This inhibitory effect of CBD was prevented by pretreatment with WAY100635 ($P=0.13$, n=4), but not AM251 ($P<0.001$, n=4). Chronic treatment with CBD (5.0 mg/kg/day for 7 days) increased the firing rate of DRN 5-HT neurons ($P<0.001$), but not the number of spontaneously active 5-HT neurons ($P=0.1$). Acute and chronic (7 days) treatment with CBD did not affect immobility ($P=0.4$, n=8) nor swimming time ($P=0.6$, n=8) in the FST in naïve rats. In contrast, chronic treatment with CBD (5.0 mg/kg/day) prevented mechanical allodynia ($P<0.05$) and thermal hyperalgesia ($P<0.05$) after 3, 7 and 14 days post-SNI in CD1 mice. **Conclusion**: These data confirm that acute CBD

administration decreases the firing rate of 5-HT neurons via the 5-HT_{1A} receptor stimulation and increases the firing rate of 5-HT neurons after 7 days of treatment. This regimen did not affect depressive behavior, but was effective in preventing allodynia and hyperalgesia associated with a neuropathic pain condition.

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